

THE EFFECTS OF FERMENTATION AND DRYING METHODS OF THEOBROMA
CACAO ON QUALITY AND FLAVOR CHARACTERISTICS

A THESIS SUBMITTED TO THE GRADUATE DIVISION OF THE
UNIVERSITY OF HAWAII AT MĀNOA IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

IN

TROPICAL PLANT AND SOIL SCIENCE

2019

By

Colin Kalani Hart

THESIS COMMITTEE

Alyssa Cho, Chairperson

Harry C. Bittenbender

Marisa Wall

Keywords: Cacao, chocolate, specialty cacao, specialty chocolate, fermentation, drying, post-harvest processing, cotyledon, testa, artificial drying, natural drying, turning protocol, organoleptics

ABSTRACT

Cacao growers in Hawai‘i face many challenges related to post-harvest processing. This is primarily due to a lack of industry-accepted standards for fermentation and drying, which are critical stages of flavor development within the bean. Farmers in Hawai‘i have expressed interest in adopting alternative processing methods to improve the quality and marketability of their product. The objective of this study was to evaluate three standard turning intervals for box fermentations, as well as 12 different drying treatments, for their effects on postharvest parameters and quality characteristics of chocolate. Fermentation F211, which was turned initially after 48 hours, followed by 24-hour intervals until completion. Protocols for fermentation and drying were considered best practice for Hawaii. Temperature readings of the top, middle, and bottom layers of each treatment were taken at ten-minute intervals throughout the fermentation cycle. pH values of the cotyledon were measured before and after fermentation, and after drying. Color attributes ($L^*C^*H^*$) were measured after drying. Sensory evaluation of chocolate, made from dried bean samples of each treatment, were conducted in two parts: evaluation of various flavor intensities (quantitative), and overall preference scores (qualitative). Chocolate samples made from each treatment were compared to a Ghanaian chocolate sample (GS), which acted as a control for flavor. Treatments were conducted monthly over an eight-month period and volume of each fermentation treatment was 227 kg. None of the response variables were shown to have interactions with season. Mean pH of the cotyledon before fermentation was 5.90. pH values decreased among all treatments during fermentation. F222 had the lowest mean among treatments, post fermentation and drying, with values of 4.37, and 5.03, respectively. F222 took significantly longer than F111 to reach the critical fermentation temperature of 43.5 °C, for each layer of the fermentation mass, but there were no differences in

mean times spent at or above this temperature between treatments. Mean maximum temperatures were significantly higher for F111 throughout each layer, although there were no differences between treatments in the time it took to reach maximum temperatures. Results from the sensory evaluation by Dandelion Chocolate showed that F222 had the highest score for fresh fruit intensity, and that GS received the highest spice intensity score. There were also differences in mean overall preference scores among treatments. F222, and F211 were scored most favorably, whereas F111, and GS scored least favorably among evaluators. F222 was shown to have consistent levels of mold infestation during fermentation, especially in the bottom corners of the mass. Therefore F211, although receiving a slightly lower preference score, could be recommended to growers as a more reliable alternative to maintain quality.

This study also examined the drying behavior of fermented cacao beans subjected to 12 different drying treatments which were categorized by heat source: 1) sun drying; 2) oven drying; and 3) dehumidification drying. Sun drying treatments were conducted at four different sites on Hawai'i Island (Pāpai'kou, Pepe'ekeo, Kainaliu, and Kawaihae) that represent a gradient of decreasing humidity and rainfall. Mechanical oven drying, and dehumidification drying were both conducted in controlled indoor environments in Hilo. Frequency of the drying interval was either constant or intermittent at each drying location. Moisture content was measured twice daily throughout the drying period. Color attributes and pH were measured before and after drying. Bean samples from each treatment were sent to Dandelion Chocolate Company for an in-depth sensory evaluation of chocolate made from each sample. Response variables were not shown to have an interaction with season. Initial mean moisture content of beans was $54.9 \pm 2.5\%$ wb. There were significant differences in mean drying rates between treatments, with sun drying

taking 17.9 days at Pāpai‘kou (the highest humidity site) and only 2.9 days for a constant oven drying treatment (ODC). Mean starting pH for the testa and cotyledon was 4.54 and 4.7, respectively. A constant sun drying treatment in Pepe‘ekea (SDPC) had the highest pH value for the testa and cotyledon, at 6.2 and 5.6 respectively. Whereas a constant dehumidification drying treatment (DDC) had the lowest testa pH (5.5), and ODC had the lowest cotyledon pH (4.8). Results from sensory evaluations indicated that an artificial intermittent treatment (ODS) had the highest rating for fresh fruit intensity, whereas the control (GS) and DDS had by far the lowest. Herbal/floral intensity ratings also differed between treatments, with the control having the highest rating, and a natural intermittent treatment (SDKS) having the lowest rating. Mean overall preference scores were not shown to differ between treatments.

ACKNOWLEDGMENTS

I would like to thank, Marc Meisner, Nick Yamauchi, Russell Galanti, and Javier Mollinedo for their extensive help in collecting data and installing treatments. I would like to thank Tom Sharkey, Andrea Kawabata, Jeff Clark, and Susan Bassett for their support in allowing me to use their facilities to set up drying and fermentation treatments. I would like to thank Dan O'Doherty for sharing his extensive knowledge of cacao with me and for his pragmatic advice in developing worthwhile treatments. I would like to thank Ed Seguire, Gary Guittard, and all the folks at Dandelion Chocolate Company, specifically: Greg D'Alesandre, Karen Cogan, and Ron Sweetser, for their indefatigable help in processing and evaluating all of the cacao samples for this project. I would like to thank Dan Corson, Berndt Stugger, Kazemaru Yukawa-Bacon, and Jim Rynders for supplying me with excellent quality pods and wet seed for my research. I would like to thank my committee chair, Alyssa Cho for advising me, providing me with funding, and for her sharp pragmatism, limitless tolerance, and unyielding support throughout this project. I would like to thank Skip Bittenbender and Marisa Wall for assisting me in experimental design, and for providing me with helpful guidance. I would like to thank Patrick Hart for sharing his extensive knowledge in data analysis and statistical design. I would like to thank the University of Hawaii system, as well as the faculty and staff at both Komohana Research and Extension center, and Kona Research Station, specifically Kathy Aoki, Eunice Domingo, and Susan Takahashi.

TABLE OF CONTENTS

ABSTRACT	2
ACKNOWLEDGEMENTS	5
LIST OF FIGURES	7
LIST OF TABLES	8
LIST OF ABBREVIATIONS	9
1. INTRODUCTION AND LITERATURE REVIEW	10
1.1 Introduction	10
1.2 Literature Review	13
1.2.1 Fermentation	13
1.2.2 Drying	15
1.2.3 Natural Drying	16
1.2.4 Artificial Drying	17
1.2.5 Quality Implications for Cacao and Chocolate	18
1.3 Summary	21
1.4 References	22
2. EVALUATION OF FERMENTATION PROTOCOLS OF CACAO BEANS ON POSTHARVEST AND CHOCOLATE QUALITY CHARACTERISTICS	24
2.1 Abstract	24
2.2 Introduction	25
2.3 Materials and methods	27
2.3.1 Site	27
2.3.2 Harvesting and cracking	28
2.3.3 Pulp pre-conditioning	28
2.3.4 Loading fermentation boxes and implementing turning protocols	29
2.3.5 Drying and storing the samples	30
2.3.6 Environmental data	31
2.3.7 pH data	31
2.3.8 Color data	31
2.3.9 Blending samples	32
2.4 Organoleptic evaluations by Dandelion Chocolate	33
2.4.1 Processing samples into chocolate	33
2.4.2 Sample preparation and evaluation methods	33
2.4.3 Intensity scaling methods	34
2.4.4 Preference scaling methods	35
2.4.5 Statistical analysis	35

2.5 Results and discussion	36
2.5.1 pH	36
2.5.2 Fermentation temperature	37
2.5.3 LCH	40
2.5.4 Flavor intensity scores	40
2.5.5 Overall preference scores	41
2.6 Conclusion	44
2.7 Tables and figures	46
2.8 References	56
 3. THE EFFECTS OF ARTIFICIAL AND NATURAL DRYING SYSTEMS ON POSTHARVEST PARAMETERS AND QUALITY CHARACTERISTICS	 58
3.1 Abstract	58
3.2 Introduction	60
3.3 Materials and methods	64
3.3.1 Site	64
3.3.2 Harvesting and cracking	64
3.3.3 Pulp pre-conditioning	65
3.3.4 Loading fermentation boxes	65
3.3.5 Implementing drying protocols	66
3.3.6 Drying treatments	67
3.3.7 Environmental data collection	68
3.3.8 Moisture data	68
3.3.9 pH data	69
3.4.1 color data	69
3.4.2 Blending	70
3.4.3 List of samples sent for evaluation	70
3.5 Organoleptic evaluations by Dandelion Chocolate	71
3.5.1 Processing samples into chocolate	71
3.5.2 Sample preparation and evaluation methods	71
3.5.3 Intensity scaling methods	72
3.5.4 Preference scaling methods	73
3.5.5 Statistical analysis	74
3.6 Results and Discussion	74
3.6.1 Drying rates	74
3.6.2 pH	76
3.6.3 LCH	77

3.6.4 Flavor intensity scores	78
3.6.5 Preference scores	78
3.7 Conclusions	79
3.8 Tables and Figures.....	81
3.9 References	91
4. CONCLUSIONS	94
4.1 Fermentation.....	94
4.2 Drying.....	95

LIST OF ABBREVIATIONS

AAB	Acetic acid bacteria.
C*	Chroma or “saturation.” Ranges from 0, which is completely unsaturated (gray, black, or white), to 60, which represents very high saturation on the LCH Color Space Model.
H°	Hue. Circular axis on the LCH Color Space Model representing saturated color, ranging from 0° (red) through 90° (yellow), 180° (green), 270° (blue) and back to 0°.
L*	Lightness. A vertical axis on the LCH Color Space Model, ranging from 0, which has no lightness (absolute black), to 100, which is maximum lightness (absolute white).
LAB	Lactic acid bacteria.
pH	Potential hydrogen. A measure of acidity or basicity in a soil solution through the measurement of hydrogen ions in the solution.
wb	Wet basis (g H ₂ O/100g FW). In the context of this thesis used to express moisture content of cacao beans.

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Hawai‘i’s cacao (*Theobroma cacao*) industry is small in comparison to cacao producing regions worldwide, yet is rapidly expanding. As Dr. Skip Bittenbender reported in a 2017 survey of the cacao industry in Hawai‘i, acreage is steadily increasing statewide at about five hectare increments per year, which is a sizeable amount considering there are only approximately 30 hectares of cacao trees that were harvested throughout the state in 2017. Because cacao is still a relatively new crop in Hawai‘i, growers are facing many obstacles in post-harvest production; which includes the fermentation and drying of the cacao seeds, (commonly referred to as beans). Fermentation and drying play critical roles in the development of flavor precursors within the bean (Afoakwa et al., 2008), and both processes require a level of expertise and experience before they can be done proficiently. Fermentation is particularly difficult in the state of Hawai‘i for several reasons: 1) there are a current lack of industry accepted standards, which has resulted in extreme variability in the final quality of fermented cacao; and 2) the majority of cacao farms throughout the state are relatively small, making it difficult for growers to attain the necessary volume required for consistent and high quality fermentations (Sukha, 2003). It is possible to address the former by implementing standard fermentation protocols that have been developed and tested in other countries. For example, studies have shown strong correlations between the timing of turning the fermentation mass and flavor characteristics of the resulting beans and chocolate (Camu et al., 2007).

The process of drying, which immediately follows fermentation, is also problematic in Hawai‘i. It is particularly difficult in the wetter areas of the state, such as the eastern regions of

Hawai‘i Island, where the rainfall and relative humidity are extremely high, and often correspond with peak harvest periods for cacao. Sun drying, which is the most commonly adopted drying method, is generally thought to produce a higher quality product than artificial drying systems (Mujaffar et al., 2017). However, the poor environmental conditions associated with east Hawai‘i make sun drying unpredictable in terms of quality. Beans will often take between 2-3 weeks to finish drying, if not longer, and will develop molds and spore forming bacteria that can lead to poor flavor and spoilage (Copetti et al., 2010). In efforts to bypass these uncontrollable environmental factors, research has been conducted on various artificial drying systems, which employ a combination of heat and airflow to dry cacao beans (Wood & Lass, 1985). The advantages of artificial drying include more control over drying rate, which can result in a quicker turnover of marketable products. However, rapid artificial drying can have adverse effects on flavor characteristics of the bean and chocolate, resulting in high concentrations of off flavors (Urquhart, 1961; Powell, 1982; Jinap et al., 1994). It is possible to offset some of these negative associations by: 1) implementing resting periods throughout the drying cycle, referred to as intermittent drying (Oke & Omotayo, 2011); and 2) ensuring that drying temperatures do not exceed 65°C during the initial drying period so as to avoid off flavors, caused in part by high levels of acetic acid concentrations (McDonald et al., 1981).

The development and implementation of processing methods that will increase the quality and consistency of Hawai‘i-grown cacao is crucial to its success as a statewide industry. Hawai‘i cannot compete with bulk cacao producers worldwide due factors including high cost of land and labor, but it can enter the specialty cacao market which focuses on the production of high-quality cacao and chocolate, rather than on sheer quantity.

The purpose of this thesis is to provide an evaluation of several fermentation and drying protocols for cacao growers in Hawai‘i, and to expand upon previous research done on these subjects. The goal of this study was to assess the impacts of various post-harvest processing methods on overall flavor characteristics. The first objective was to evaluate the effects of three different fermentation methods on pH and color attributes of the bean, and on the quality characteristics of the resulting chocolate. The second objective was to determine the effects of natural and artificial drying systems on pH, color attributes, and drying rate of cacao beans, as well as flavor characteristics of the chocolate through sensory evaluation.

The hypotheses for each objective are as follows. For objective one, fermentation treatments are expected to have a positive effect on pH, color, as well as for flavor intensities, and overall preference scores in comparison to the Ghanaian samples (GS), which acted as a control. F111 will produce beans of higher quality compared to other treatments. This will be apparent through an increase in pH, quicker rate in reaching and sustaining critical fermentation temperatures, and an increase in color attributes. The second objective will test several hypotheses. Drying treatments will have an effect on pH, color, drying rate, flavor – intensities, and overall preference scores over the control. Sun-drying treatments in Pāpai‘kou and Pepe‘ekeo are expected to take longer to dry than the other treatments. Oven dried treatments and dehumidification treatments will produce beans with lower pH in comparison to the sun drying treatments, and the respective chocolates will have higher levels of off flavors.

The duration of this study was 1.5 years, from treatment application on September 2017 to December 2018. Final rounds of flavor evaluations will be completed in July 2019.

1.2 Literature Review

1.2.1 Fermentation

Fermentation is perhaps the most critical stage in the post-harvest processing of cacao as it initiates the development of principal flavor precursors within the bean that are necessary in the production of high quality chocolate (Afoakwa et al., 2008; Hernandez et al., 2015; Schwan & Wheals, 2014). There are two primary methods of fermentation that are used throughout the cacao producing world: heap fermentations, and box fermentations (Rohan, 1963; Wood & Lass, 1985), the latter being more commonly associated with high quality cacao, as well as being the standard practice in Hawai‘i, and is therefore the focal point of this study. Under ideal conditions, box fermentations are conducted within covered and partially enclosed areas, and consist of thick hardwood boxes with small holes drilled in the bottom, to facilitate the drainage of pulp, caused by the degradation of pectin by microbial pectinase, throughout the fermentation (Are & Gwynne-Jones, 1974; Wood & Lass, 1985; Ouattara et al., 2008). The beans are covered with a thick, yet lightly packed layer of banana leaves and burlap to assist in the retention of moisture and heat throughout the 6-8 day fermentation period (Wood & Lass, 1985). The beans are manually turned from one box to another at regular intervals. There are various protocols relating to the timing of turning the fermentation mass. The most common protocols involve either turning every 24 hours, or every 48 hours, depending on the producer (Wood & Lass, 1985). This process not only ensures uniformity in the fermentation mass, but also initiates the activity and succession of the various microorganisms, such as acetic acid-forming bacteria, that are responsible for flavor development within the bean (Camu et al., 2008; Biehl, 1982).

The microbial succession of cacao fermentation is well documented (Passos et al., 1984; Schwan, 1998; Holben et al., 2004; Schwan & Wheals, 2004; Papalexandratou et al., 2011). The

first 48 hours of fermentation are dominated by various species of yeast, which are naturally occurring on the sugar-rich, mucilaginous pulp of the bean after the pod is opened and the beans removed (Ardhana & Fleet, 2003), and convert sucrose, glucose, and fructose into ethanol and CO₂ (Schwan & Wheals, 2004; Afoakwa et al., 2008). It is during this period that citric acid is metabolized, which causes the pH of the pulp to rise and make a more hospitable environment for bacterial growth, specifically lactic acid-forming bacteria (LAB) and acetic acid-forming bacteria (AAB).

After the first two days of anaerobic fermentation, the mass is generally turned for the first time, exposing the beans to oxygen, and thus initiating the aerobic stage of fermentation. AAB become more active under aerobic conditions and proceed to oxidize ethanol into acetic acid (Campos et al., 2011; Schwan & Wheals, 2004), which is a key metabolite in the fermentation process (Camu et al., 2008). This process is extremely exothermic, and causes a rapid temperature spike in the fermentation mass, often reaching approximately 50°C or higher (Knapp, 1937). Concurrently, acetic acid diffuses into the cotyledon, resulting in a decrease in pH (Thompson et al., 2001; Campos et al., 2011) and an activation of hydrolytic enzymes that could potentially result in the creation of various flavor compounds in the bean (Schwan & Wheals, 2004; Campos et al., 2011).

The aerobic phase also marks a series of oxidative reactions in cyanidin and protein-phenolic complexes, which leads to noticeable color changes on the surface of the cotyledon (Afoakwa, 2016). The browning in color of the cotyledon, in combination with the sharp aromas of volatilizing acetic acid, are both parameters in determining the completion of fermentation (Wood & Lass, 1985). Aerobic spore forming bacteria may also develop towards the end of the fermentation, especially on the edges and bottoms of the fermentation box where there is less

moisture and heat (Maravalhas, 1966; Schwan et al., 1995; Senenayak et al., 1997; Camu et al., 2008).

1.2.2 Drying

Drying is an extension of the fermentation process, and must be done to effectively reduce the moisture content of the bean from approximately 60% to 6-8% moisture (Cunha, 1990), and to promote the outward migration of volatile acidity, primarily acetic acid, which diffused into the cotyledon during fermentation. Drying allows many of the biochemical changes that were initiated in the fermentation process to continue and further promote flavor development.

The rate of drying is critical to final quality (Sukha, 2003). If beans are dried too quickly it can result in case hardening, a process by which volatile acidity is trapped within the cotyledon, resulting in beans with high levels of bitterness and acidity. However, if the beans dry too slowly, they run the risk of developing molds and off flavors, and can spoil in storage (Wood & Lass, 1985; Afoakwa et al., 2008; Sukha, 2003).

Previous studies by Bravo & McGraw (1974), Duncan et al. (1989), and Fagunwa (2009), identified three distinct stages in the drying rate of cacao: a constant rate, followed by a first and second stage falling rate. The constant rate of drying, which occurs from the starting moisture percentage of the bean ($\approx 60\%$) to about 40% moisture, involves the transfer of water from the testa into the surrounding air. The transfer of water during this stage is equally dependent on ambient temperature and the rate of airflow. The first stage falling rate, which occurs from about 40% to 23% moisture, is where the water occupying the layer between the cotyledon and testa is removed, proceeded by the second stage falling rate, which occurs from about 23% to 6-8% moisture, where water is diffused from the cotyledon to the testa, and is then evaporated into the

atmosphere. The two falling rate stages are less dependent on airflow, and more on the temperature of the ambient air (Wood & Lass, 1985; Shelton, 1967).

1.2.3 Natural Drying

There are two main systems of drying: natural, and artificial. Natural drying, or sun drying, is a system that uses a combination of solar radiation and natural airflow to dry beans, and is by far the most widely adopted method of drying worldwide (Wood & Lass, 1985). Sun drying is most commonly used because it is inexpensive and thought to be best for optimal quality (Fagunwa et al., 2009; Sukha, 2003). There are many variations within this system, although the most prominent involve either drying beans on concrete or wooden floors, or on raised drying screens within a partially enclosed structure or greenhouse. In some regions, such as the West Indies, beans are dried on lifted wooden platforms with a movable roof that can be moved over them during times of inclement weather (Mujaffar et al., 2017). Throughout the drying period beans must be stirred frequently to prevent uneven drying and to break apart aggregates (Hart, 1900). It is especially important to stir the beans during the first few days of drying to reduce the potential of over-fermentation caused by inadequate heat and airflow (Thien & Yap, 1994).

1.2.4 Artificial drying

Artificial drying is often implemented in regions where high rainfall and humidity make drying difficult (Asiedu, 1989). Artificial drying involves the use of heat exchangers or direct-fired heaters to decrease drying rate of cacao (Wood & Lass, 1985). There are many design variations within this system, although the majority of them employ the combination of heat and airflow to

dry cacao beans. The advantages of artificial drying include control over drying rate, which can result in a quicker turnover of marketable products.

However, speed of drying should not be the only factor of concern for the development of a marketable product. There have been multiple studies on the effects of rapid artificial drying on flavor characteristics of cacao and chocolate. A 1994 study by Jinap et al. observed case hardening under rapid artificial drying conditions, which resulted in high levels of off flavors, including astringency and bitterness, in beans that were oven-dried at 60°C, in comparison to beans that were sun-dried, which were scored favorably by the tasting panelists. Conversely, studies done by de Vos (1956), and by Howat et al. (1957), reported no differences in the flavor of chocolate between beans dried rapidly for 11-14 hours, and sun-dried beans. However, it was noted in both studies that there were higher levels of acid flavors in the rapidly dried beans, compared to the sun-dried beans, but that it was removed during conching of the chocolate. This supports the hypothesis of case hardening under rapid artificial drying, but suggests that certain levels of acidity in the bean can be removed during processing.

Some studies have shown that implementing rest periods during the drying cycle (commonly referred to as intermittent drying) can reduce the negative implications associated with rapid drying. For example, Oke & Omotayo (2011), found no adverse effect on quality from beans dried intermittently in a forced air artificial system. Similarly, a producer in Trinidad who implemented artificial intermittent drying on his farm reported that chocolate makers could not tell the difference in quality between artificially dried beans, and beans dried under natural sun drying conditions (Pers. Comm., Daniel O'Doherty, February 13, 2017). The purpose of intermittent drying is to allow the beans to have a resting period, which stimulates the migration

of moisture and volatile acidity within the cotyledon, to the testa, and then into the surrounding air (Wood & Lass, 1985).

1.2.5 Quality implications of cacao and chocolate

The Model Ordinance of the International Cocoa Standards states that cacao of merchantable quality must be: “(a) *Fermented, thoroughly dry, free from smoky beans, free from abnormal or foreign odours and free from any evidence of adulteration.* (b) *Reasonably uniform in size, reasonably free from broken beans, fragments and pieces of shell, and virtually free from foreign matter.*”

There are many parameters in place for measuring quality of fermented cacao beans, including: moisture content, bean count (amount of beans required to make up 100g), flavor quality, and the cut-test (Afoakwa, 2016). The cut-test is perhaps the most widely adopted of these methods because it is simple to perform, and effective in identifying physical characteristics associated with general quality (Lopez & Dimick, 1995; Guehi et al., 2007). The International Organization for Standards (ISO) requires that a minimum of 300 beans must be checked, using the cut-test method, for every ton of cacao. The cut-test is performed by slicing a sample of fermented and dried cacao beans longitudinally, and visually evaluating the cotyledon for various indicators, including: beans that are flat and shrunken, moldy, slaty (ble to gray color and unfermented), germinated, or infested with insects and/or rodents, all of which can drastically impact flavor (Wood & Lass, 1985, Fagunwa, 2009; Afoakwa, 2016). International standards require that marketable cacao beans should not exceed the following levels of defects based on the cut-test: 3% moldy beans, 3% unfermented beans, 3% insect or rodent damaged beans, or 3% germinated or flat beans, by count.

The cut-test is effective in determining general flavor defects in cacao beans, i.e. excessive bitterness or astringency caused by high proportions of slaty beans, but is not a reliable indicator of flavor quality (CAOBISCO/ECA/FCC, 2015). Flavor quality is a key criterion used to determine overall quality characteristics of cacao and chocolate (Afoakwa, 2016), although it is used less frequently than the cut-test because it is time consuming and inherently more subjective. Flavor quality involves processing the beans into cocoa-liquor or chocolate, and using sensory evaluation to determine desirable flavor profiles, as well as flavor defects. However there is currently a lack of credible and verifiable protocols for evaluating quality attributes of cacao and chocolate, which has led to extreme variability in research that includes flavor quality as a method of evaluation. This is currently a major topic of interest among industry members. For example, in 2018 members of the Working Group (WG) on the development of International Standards for the Assessment of Cocoa Quality and Flavor (ISCQF) drafted and reviewed 20 protocols to be used as standardized methods for accurately and consistently evaluating cacao and chocolate. Participants of the WG include: Barry Callebaut, Centre for the Promotion of Imports from developing countries (CBI), USAID-Equal Exchange – Tcho Cooperative Development Programme (DCP), Cocoa of Excellence Programme (CoEx), International Cocoa Awards (ICA), Cocoa Research Centre of the University of the West Indies (CRC), Fine Cacao and Chocolate Institute (FCCI), Heirloom Cacao Preservation (HCP), Guittard Chocolate Company, Lutheran World Relief (LWR), and Penn State University (PSU), among others. Each of these entities has done individual work on the subject of quality assessment for cacao and chocolate, which were used as reference points in the ISCQF proposal.

Recent steps towards the reevaluation of quality assessments indicate a shift in the overall perspective regarding the importance of flavor quality. It is only logical that as changes are made

in post-harvest processing methods to highlight the complex flavor nuances in the production of high quality chocolate, so too should the systems that are used to evaluate them.

1.3 Summary

Developing standardized post-harvest processing methods that will increase the quality and consistency of Hawai'i-grown cacao and chocolate is necessary in securing its success as a viable statewide industry. Previous research has shown the importance of post-harvest systems, such as fermentation and drying, on numerous quality characteristics in the cacao bean and resulting chocolate, and has thus provided a justification for further research to be conducted. This thesis is divided into two primary chapters. The first is focused on the effects of various fermentation protocols on temperature profile, pH, color attributes, and quality characteristics of the cacao beans and chocolate. The second is on evaluating the effects of different artificial and natural drying treatments on rate of moisture loss, pH, color attributes, and quality characteristics of the resulting cacao beans and chocolate.

1.4 References

- Afoakwa, E.O., A. Paterson, M. Fowler, and A. Ryan. 2008. Flavor information and character in cocoa and chocolate: A critical review. *Critical Reviews in Food Science and Nutrition* 48:840-857.
- Afoakwa, E.O. 2016. *Cocoa Production and Processing Technology*. Boca Raton, FL: Taylor & Francis Group.
- Ardhana, M.M., and G.H. Fleet. 2003. The microbial ecology of cocoa bean fermentations in Indonesia. *International Journal of Food Microbiology*, 86:87-99.
- Are, L.A. and D.R.G. Gwynne-Jones. 1974. Harvesting and processing of Cocoa. In *Cacao in West Africa*. Oxford University Press, Ibadan, Nigeria.
- Asiedu, J.J. 1989. *Processing Tropical Crops: A Technological Approach*. Macmillan Press Limited, London, pp. 24-41.
- Biehl, B., D. Passern, and W. Sagmann. 1982. Effect of acetic acid on subcellular structures of cocoa bean cotyledons. *J. Sci. Food Agric.* 33: 1101-1109.
- Bravo, A. and D.R. McGaw. 1974. Experimental artificial drying characteristics of cocoa beans. *Trop. Agric., Trin.* 51: 395-506.
- CAOBISCO/ECA/FCC. 2015. “Cocoa Beans: Chocolate and Cocoa Industry Quality Requirements”. Appendix B “Protocols for the preparation and flavour evaluation of samples and small-scale fermentation techniques” – D. Sukha and E. Seguire. (End, M.J. and Dand, R., Editors).
- Camu, N.T., K.S. De Winter, J.S. Addo, J.F. Takrama, H. Bernaert, and L. De Vuyst. 2008. Fermentation of cocoa beans: Influence of microbial activities and polyphenol concentrations on the flavor of chocolate. *J. Sci. Food Agric.* 88: 2288-2297.
- Copetti, M.V., J.L. Pereira, B.T. Iamanaka, J.I. Pitt, and M.H. Taniwaki. 2010. Ochratoxigenic fungi and ochratoxin A in cocoa during farm processing. *Intl. J. Food Microbiol.* 143: 67-70.
- Cunha, J.D. 1990. Performance of Burairo 3x3m dryer for cocoa. *Agrotropica*, 2(3): 157-160
- ICCO 2004. *Cocoa statistics*, 29 (3).

Duncan, R.J.E., G. Godfrey, T.N Yap, G.L. Pettiphar, and T. Tharumarajah. 1989. Improvement of Malaysian cocoa bean flavor by modification of harvesting, fermentation, and drying methods – The Sime Cadbury Process. *The Planter*, Kuala Lumpur 65: 157-173.

Fagunwa, A.O., O.A. Koya, and M.O. Faborode. 2009. Development of an intermittent solar dryer for cocoa beans. *Agricultural Engineering International: CIGR Journal*.

Fowler, M.S. 2009. Cocoa beans: From tree to factory. In *Industrial Chocolate Manufacture and Use*, 4th edition. S.T. Beckett (Ed.). Oxford: Blackwell. Pp. 10-33, 137-152.

Guehi, T.S., Y.M. Konan, R. Koffi-Nevry, N.D. Yao, and N.P. Manizan. 2007. Enumeration and identification of main fungal isolates and evaluation of fermentation's degree of Ivorian raw cocoa beans. *Australian Journal of Basic and Applied Sciences*, 1(4): 479-486.

Hart, J. H. 1900. *Cacao. A treatise on the cultivation and curing of 'cacao' (2nd edn.)* Mirror, Port-of-Spain, Trinidad.

Howat, G. R., Powell, B.D. and Wood, G. A. R. 1957b. Experiments on cocoa drying and fermentation in West Africa. *Trop. Agric., Trin.* 34: 249-59.

Knapp, A. W. 1937. *Cocoa Fermentation: A Critical Survey of Its Scientific Aspects*. Bale and Curnow, London, 171p.

Lopez, A. S. and Dimick, P.S. 1995. Cocoa fermentation. In: *Biotechnology: A Comprehensive Treatise*, vol. 9, Enzymes, Food, and Feed pp. 563-577. Reed, G. and Nagodawithana, T.W., Eds. (2nd ed.). VCH, Weinheim.

Maravalhas, N. 1966. Microbiological deterioration of cocoa beans during fermentation and storage in Bahia. *Revue Internationale de la Chocolaterie*, 21:375-378.

McDonald, C.R., Lass, R.A., Lopez, A.S. 1981. Cocoa drying – A review. *Cocoa Growers' Bulletin*, 31:5-39.

Mujaffaer, S., Sukha, D. A., Ramroop, A. 2017. Comparison of the drying behavior of fermented cocoa (*Theobroma cacao* L.) beans dried in a cocoa house, greenhouse and mechanical oven. Cocoa Research Centre, The University of the West Indies, St. Augustine, Trinidad.

Oke D.O., and Omotayo K.F. 2012. Effect of forced-air artificial intermittent drying on cocoa beans in outh-Western Nigeria. Department of Agriculture and Bio-environmental Engineering. School of Engineering. Nigeria.

Ouattara, H.G., B.L. Koffi, G.T. Karou, A. Sangare, S.L. Niamke, and J.K. Diopoh. 2008. Implication of *Bacillus* spp. In the production of pectinolytic enzymes during cocoa fermentation. *World Journal of Microbiology and Biotechnology* 24:1753-1760.

- Papalexandratou, Z., Falony, G., Romanens, E., Jimenez, J.C., Amores, F., Daniel, H-M., De Vuyst, L. 2011. Species diversity, community dynamics, and metabolite kinetics of the microbiota associated with traditional Ecuadorian spontaneous cocoa fermentations. *Applied and Environmental Microbiology*, 77:7698-7714.
- Passos, F.M.L., Silva, D.O., Lopez, A., Ferreira, C.L.L.F., and Guimares, W.V. 1984. Characterization and distribution of lactic acid bacteria from traditional cocoa bean fermentations in Bahia. *J. Food Sci.*, 49: 205-208.
- Powell, B.D. 1982. The quality of cocoa beans – The needs of the manufacturer. In *Proceedings of the 8th International Cocoa Research Conference held in Cartagena, Colombia*, pp. 755-758.
- Rohan, T. A. 1963. Preparation of Raw Cocoa for the Market. *FAO Agric. Studies No. 60*. Rome.
- Schwan, R. F., Rose, A.H., Board, R.G. 1995. Microbial fermentation of cocoa beans, with emphasis on enzymatic degradation of the pulp. *Journal of Applied Bacteriology (Supplement)*, 79: 96S-107S
- Schwan, R.F. 1998. Cocoa fermentations conducted with a defined microbial cocktail inoculum. *Applied and Environmental Microbiology* 64:1477-1483.
- Schwan, R. F. and Wheals, A. E. 2004. The microbiology of cocoa fermentation and its role in chocolate quality. *Crit. Rev. Food Sci. Nutr.* 44:205-221.
- Senenayake, M., Jansz, E.R., Buckle, K.A. 1997. Effect of different mixing intervals on the fermentation of cocoa beans. *Journal of the Science of Food and Agriculture*, 74: 42-48.
- Shelton, B. 1967. Artificial drying of cocoa beans. *Tropical Agriculture*, 44:125-32
- Sukha, D. A. 2003. Primary processing of high quality Trinidad and Tobago cocoa beans-targets, problems, options. *Cocoa Research Unit, University of the West Indies, St. Augustine Trinidad*.
- Thien, J. and T.N. Yap. 1994. Effect of drying on acidity and volatile fatty acids content of cocoa beans. *Journal of Science Food Agriculture*, 67-75.
- Thompson, S.S., K.B. Miller, and A.S. Lopez. 2001. Cocoa and coffee. In *Food Microbiology – Fundamentals and Frontiers*. M.J. Doyle, L.R. Beuchat, and T.J. Montville (Eds.). Washington DC: ASM Press, pp. 721-733.
- Urquhart, D.H. 1961. *Cocoa*, 2nd edn. Western Printing Services Ltd., Bristol, Great Britain.
- De Vos, L. (1956) Artificial drying of cocoa. *Bull.* 73, Landouwproef Station in Suriname.
- Wood, G.A.R., and R.A. Lass. 1985. *Cocoa*, 4th edition. London, UK: Longman Group.

CHAPTER 2

EVALUATION OF FERMENTATION PROTOCOLS OF CACAO BEANS ON POSTHARVEST AND CHOCOLATE QUALITY CHARACTERISTICS

2.1 Abstract

Cacao growers in Hawai'i face many challenges related to post-harvest processing. This is primarily due to a lack of industry-accepted standards, especially concerning fermentation, which is a critical stage for flavor development within the bean. The objective of this study was to evaluate three standard turning intervals for box fermentations in Hawai'i, and their effects on postharvest parameters and quality characteristics of chocolate. Treatments included: Fermentation 1-1-1 (F111), which was turned every 24 hours, Fermentation 2-2-2 (F222), which was turned every 48 hours, and Fermentation 2-1-1 (F211), which was turned initially after 48 hours, and then at 24-hour intervals until completion. Fermentation periods were seven days for each treatment. Temperature readings of the top, middle, and bottom layers of each treatment were taken at ten-minute intervals throughout the fermentation cycle. The pH values of the cotyledons were measured before and after fermentation, and after drying. Color attributes ($L^*C^*H^*$) were measured after drying. Sensory evaluation of chocolate made from dried bean samples of each treatment, were conducted in two parts: evaluation of various flavor intensities (quantitative), and overall preference scores (qualitative). Chocolate samples made from each treatment were compared to a Ghanaian chocolate sample (GS), which acted as a control for flavor. Treatments were conducted over an eight-month period, and replicated over time. None of the response variables were shown to have interactions with season. Mean pH of the cotyledon before fermentation was 5.90. The pH values decreased among all treatments during fermentation. Treatment F222 had the lowest mean pH both post fermentation (4.37) and after

drying (5.03). F222 took significantly longer than F111 to reach the critical fermentation temperature of 43.5 °C, for each layer of the fermentation mass, but there were no differences in mean times spent at or above this temperature between treatments. Mean maximum temperatures were significantly higher for F111 throughout each layer, although there were no differences between treatments in the time it took to reach maximum temperatures. Results from the sensory evaluation by Dandelion Chocolate showed that F222 had the highest score for fresh-fruit intensity, and that GS received the highest spice intensity score compared to all other treatments. There were differences in mean overall preference scores among treatments. F222 and F211 were scored most favorably, whereas F111 and GS scored least favorably among evaluators. F222 was shown to have consistent levels of mold infestation during fermentation, especially in the bottom corners of the mass. Therefore F211, although receiving a slightly lower preference score, could be recommended to growers as a reliable fermentation protocol for East Hawai‘i.

2.2 Introduction

Cacao (*Theobroma cacao*) has only recently been considered in Hawai‘i as a potentially viable specialty crop, and therefore faces many challenges in establishing the necessary standards of quality for it to succeed as a competitive statewide industry. The production of Hawai‘i-grown cacao and chocolate is limited by the high price of land and labor, in comparison to other cacao producing regions worldwide, and is therefore dependent on producing a high quality product, rather than sheer quantity. However, there are many obstacles relating to post-harvest processing of cacao in Hawai‘i. The process of fermentation, which begins after harvesting and opening the pods, is among this set of challenges, and due to its substantial role in

flavor development within the bean is perhaps the most crucial to properly manage (Wood and Lass, 1985; Schwan & Wheals, 2004; Aculey et al., 2010).

Fermentation is particularly difficult in Hawai‘i for several reasons, primarily because there is a lack of industry accepted standards that growers can follow, which has resulted in extreme variability in quality. It is possible to address this problem by implementing common fermentation protocols that have been developed and tested in other countries, and that have been met with a certain level of success. However, it is important to evaluate site-specific effects of standardized fermentation methods, because there has been little to no research formally conducted on this subject for Hawai‘i-grown cacao.

A key component of fermentation is the interval at which the fermentation mass is aerated, or “turned”. Turning protocols vary depending on the producer (Senenayake, 1997), although the most common methods involve turning at intervals of 24 hours or 48 hours throughout the fermentation period, which is generally 6-8 days (Wood & Lass, 1985). Turning is done by manually scooping the beans from one container into the next. This process promotes uniformity in the fermentation mass, and initiates the activity of aerobic microorganisms, primarily acetic acid bacteria (AAB), that are responsible for temperature fluctuations within the fermentation, and that contribute to flavor development in the cotyledon (Camu et al., 2008).

Turning intervals have been the focus of many studies due to their critical impact on post-harvest parameters, such as pH, rate of temperature increase, and quality characteristics, including flavor (Guehi, 2010; Passos, 1984; Senanayake, 1997; Camu, 2008). However, amongst these studies are a wide range of experimental variables, including the genetic material of the cacao used, length of time between harvesting and fermenting, volume of starting material, method of fermentation (i.e., box or heap), length of fermentation, time between turning, and

sensory evaluation protocols used to determine quality. This has resulted in a relatively limited understanding of “best practice” for cacao fermentation. However, it should be noted that the majority of high quality cacao that is currently purchased by craft chocolate makers worldwide, share some similar characteristics in terms of processing methodology. Pods are generally cracked within 48 hours of harvesting, beans are fermented in wooden boxes, fermentation volumes often exceed 200 kg, the length of fermentation ranges from five to seven days, and the fermentation mass is frequently turned, using some variation on either a 24 hour or 48 hour interval (Pers. Comm., Daniel O’Doherty, June 13, 2019). These similarities in processing methods, which have been adopted by reputable cacao producers, indicate some foundational protocols that could be used to develop standardized fermentation methods in Hawai‘i.

The objective of this study was to evaluate the effects of various fermentation turning protocols on post-harvest parameters, including temperature profile, pH, color attributes, and sensory evaluations.

2.3 Materials and methods

2.3.1 Location

Field experiments were conducted monthly between September 2017 and April 2018 (8 months). Ripe cacao pods were obtained from five separate smallholder farms located along the district of North Hilo on Hawai‘i island. Elevation above sea level for each farm ranged from 72 meters to 244 meters. Average annual rainfall for this district varies from 3,300 mm to 5,000 mm.

Fermentation trials were done within a partially enclosed greenhouse at Hilo Shark's Chocolate Farm, located in Pāpai'kou, on the eastern region of Hawai'i Island. Ambient conditions within the greenhouse, including temperature and relative humidity (RH), are reported in Figure 2-1.

Post fermentation, the beans were sun dried in a partially enclosed greenhouse at the Kona Research Station, located in Kainaliu, which is on the western portion of Hawai'i Island. Ambient conditions within the greenhouse during monthly drying periods are reported in Figure 2-2.

2.3.2 Harvesting and cracking

Ripe cacao pods, from seedling trees of unknown parental genetics, were harvested using hand-pruners, collected in burlap sacks, hauled from the orchard and transported by truck to a central processing area at Hilo Shark's Chocolate Farm. Pods were then unloaded into a covered area and cracked open with machetes to extract the beans. This was done by striking the pod horizontally with the machete, and then twisting the blade to leverage open the husk. The split pods were then placed in a pile where the seeds were removed from the "placenta" of the pod, and placed in five-gallon plastic containers.

2.3.3 Pulp pre-conditioning

Wet seed was immediately homogenized upon completion of cracking within a large perforated stainless-steel container to allow excess juice to drain from the beans prior to fermentation. This process, known as pulp pre-conditioning, has been reported to reduce acidity in the cotyledon (Afoakwa, 2016). Beans were left in the strainer for approximately two hours before being

transferred into fermentation boxes. The mass was stirred every thirty minutes to promote even drainage.

2.3.4 Loading fermentation boxes, and implementing turning protocols

Wet seed was removed from the strainer and loaded into three separate fermentation boxes, each corresponding to a treatment. The fermentation boxes were constructed of untreated maple plywood, measuring 0.6 cubic meters per box. Holes (10 mm) were drilled in the bottom of each box to facilitate drainage throughout fermentation. Each box held 227 kg of wet seed at approximately 90% capacity, to allow for expansion of the fermentation mass. Three replicates of subsamples consisting of 1.5 kg of wet seed in a small mesh sack were placed in the top (15 cm), middle (30 cm), and bottom (45 cm) of the fermentation mass for a total of nine subsamples (Figure 2-3). The placement of the subsamples was to ensure an accurate representation of the fermentation mass in samples that would later be subject to physical and organoleptic evaluation. Since there is often a steep temperature gradient present in box fermentations (Wood & Lass, 1985), it is necessary to include samples at various depths and locations throughout the fermentation mass to account for that variability.

Once the boxes were loaded, and subsamples installed, each box was randomly assigned to a treatment: F111, which consisted of turning or aerating the fermentation mass every 24 hours over a 7 day period; F222, which consisted of turning the mass every 48 hours over a 7 period; and F211 which involved turning the fermentation once after 48 hours and then at 24 hour intervals every day thereafter over a 7 day period. To adhere to common fermentation practices, fresh banana leaves were used to cover the surface of the fermentation mass (Figures 2-4a), followed by a thick, but loosely assembled layer of burlap sacks (Figure 2-4b) to provide insulation (Wood and Lass, 1985; Sukha, 2003). Fermentations were turned from one box to

another using a plastic grain scoop. As each fermentation mass was turned according to its protocol, the subsamples were removed from the fermentation and massaged by hand to aerate the beans. Subsamples were then placed into the next fermentation box in an inverse arrangement to their original placement. This process was repeated upon each turning cycle until the fermentation period was completed. On the fourth day of fermentation, banana leaves were placed along the bottom of each fermentation box before the beans were turned. This was done to cover the drainage holes, since most of the mucilage surrounding the beans had degraded during the fermentation process and no longer needed to drain. This practice also helps to retain moisture and heat in the bottom layer of the fermentation mass, reducing the likelihood of molds and spore forming bacteria infecting the fermentation (Pers. Comm., Daniel O'Doherty, July 3, 2017).

2.3.5 Drying and storing the samples

Upon completion of the fermentation, the nine subsamples from each treatment were removed from the fermentation box and mixed together. Samples were immediately delivered to the Kona Research Station in Kainaliu, a branch of the College of Tropical Agriculture and Human Resources (CTAHR), for drying. Samples were placed on plastic mesh screens at a depth of 5 cm and were dried in an open-air greenhouse using a procedure developed by Ed Seguire, owner of Seguire Cacao Cocoa & Chocolate Advisors, LLC: three hours of sun exposure the first day (9 am to 12 pm), four hours the second day (9 am to 1 pm), six hours the third day (9 am to 3 pm), and eight hours every day thereafter (9 am to 5 pm) until the beans reached 7-7.5 % moisture. Samples were stirred vigorously by hand every 30 minutes during the first day of drying (from approximately 9am to 5pm), every hour during the second day, every three hours the third day, and three times per day on each of the subsequent days until the beans were dry. Stirring was

done to promote even drying amongst samples (Hart, 1900). Upon completion of drying, samples were removed from their screens and placed in separate mesh bags and stored at ambient temperature and relative humidity for at least six weeks before being blended, packed, and submitted for organoleptic evaluations.

2.3.6 Environmental data

HOBO 8K Pendant Data Loggers (Onset Computer Corporation, Cape Cod, MA) were placed at the top, middle, and bottom of the fermentation mass, and were set to measure temperature at ten-minute intervals throughout the fermentation period. An Onset S-WSB-M003 Wind Speed Smart Sensor was used to calculate wind speed (m/s) at thirty-second intervals in the greenhouse. The HOBO U12-013 Data Logger was used to measure ambient temperature (°C) and relative humidity (%) within the greenhouse.

2.3.7 pH data

The pH of cotyledons and testa were taken before and after fermentation, as well as after drying, in all treatments. Samples (10 g) from each treatment of beans were manually husked to remove the testa from the cotyledon, and ground separately using a mortar and pestle. Samples were homogenized for 30 seconds in 100 ml of distilled water. A 25 ml aliquot was pipetted into a beaker and the pH was measured using an OAKTON pH 150 meter (OAKTON Instruments, Vernon Hills, IL).

2.3.8 Color data

Color of both the cotyledons and testa were measured post fermentation among all treatments, using a Minolta Chroma Meter CR-300 (Konica Minolta, Inc.). L*C*H* represent three axes on the LCH Color Space Model. The L* axis is vertical and represents Lightness: the range from absolute black to absolute white. The C* axis represents Chroma (saturation), which ranges from completely unsaturated (i.e. natural grey, white, or black) to high saturation. H* is the circular axis on the model, and represents Hue angle, ranging from 0° (red) through 90° (yellow), 180° (green), and 270° (blue). Triplicate L*, C*, and H* readings were measured for each treatment.

2.3.9 Blending samples

After samples had cured in breathable sacks for at least six weeks, they were combined within each treatment according to the season in which they were processed: Fall 2017 (n=4), and Spring 2018 (n=3), so that there was a single representative sample for each treatment per season. Blending was done for two main reasons: 1) to reduce the number of samples that the evaluators needed to process, and 2) to mimic procedures carried out in scaled production systems, where beans from different lots are blended according to quality, so as to promote uniformity in marketable bean shipments. This was done by mixing the samples, per treatment, per season, in a large stainless-steel bowl. The mixture was then sorted to remove germinated, broken, or under developed beans. A 1,600 g sample was then taken from the mixture and sealed in a one-gallon Ziploc® bag (S.C. Johnson & Son, Inc.).

Samples were packaged and sent to Dandelion Chocolate Company for sensory evaluation. The sample list included the following fermentation treatments: F111, F211, and F222, as well as a sample of Ghana-grown beans, (GS), to act as a control for quality. Ghanaian cacao is the

standard by which all cacao is measured, and is commonly used in organoleptic evaluations as a reference point in identifying key flavor components within samples (Afoakwa, 2016). The first set of samples, which represented the Fall 2017 harvest season, was sent in February 2018, and the second set, which represented the Spring 2018 season, was sent in June of 2018.

2.4 Organoleptic evaluations by Dandelion Chocolate

2.4.1 Processing samples into chocolate

Samples were processed according to a standard protocol developed by Dandelion Chocolate Company: Approximately 1600 g of cacao beans were roasted in a Behmor 1600 coffee roaster (Behmor Inc. Incline Village, NV) at 120°C for 17.5 minutes, with a 13-minute cool down period. After the beans were cracked and winnowed, 1 kg of 70% chocolate was made, consisting of 700 g of cacao nibs and 300 g of unrefined cane sugar, using a mini *mélanger*. The chocolate was ground and conched for 18-20 hours, thus reducing the particle size to approximately 25 microns. Untempered chocolate was then poured into 10 mm square molds where they were stored until the time of evaluation.

2.4.2 Sample preparation and evaluation methods

A total of seven evaluators from Dandelion Chocolate, who were essentially untrained and uncalibrated were chosen specifically for two reasons: 1) they were well practiced tasters: each of them had constant exposure to cacao beans and chocolate from a wide range of origins and quality; and 2) they were thoughtful and reliable tasters that were capable of committing to the evaluation schedule, and taking their role seriously.

Each of the seven evaluators completed two replicates of each sample per season (Fall 2017 and Spring 2018), for a total of 14 evaluations per treatment per season. Each season was a replicate for the treatments. Each taster was a subsample. All samples were blinded with two unique 3-digit blind codes that related to the replication number.

The order in which samples were tasted was randomized, however replicate samples were intentionally spread out to ensure that evaluators would not taste the same samples back to back. Evaluators used the GoCanvas application (2018 Canvas Solutions, Inc.) to directly input data.

2.4.3 Intensity scaling methods

Samples were evaluated based on the following categorical aromatic groups: Cocoa, nut, fresh-fruit, dried-fruit, dairy, sugar, spice, herbal/floral, other, and off-flavor intensities.

Due to the untrained nature of the panel, a relatively basic intensity scale was chosen:

0 - Not Present

1- Faint, but present

2 - Present, but not main note

3 - Dominant note

This scale was designed to force evaluators into making a concise decision on the intensity of detected flavors. The more commonly used 0-10 scale leaves more room for subtle differences in flavor between samples, but also creates a wider space for error when score results are combined among multiple evaluators. A Word Cloud generator (© Jason Davies) was implemented to display common words or phrases used in each flavor intensity category. This was done to better visualize the data among evaluators.

2.4.4 Preference scaling method

The Love it/Acceptable/Hate it Scale was developed to offer a sense of actual preference between samples. For example, when an evaluator scores a particular sample with a “3” for cocoa intensity and a “1” for fresh-fruit, a clear differentiation between main flavor groups is established, but a sense of overall quality is relatively untouched. This categorical scale gives the evaluator a chance to state if they actually liked the sample they just tasted, or not. Including this categorical method of evaluation in the study is important because it represents a facet of the decision-making process that industry professionals use when purchasing cacao from various origins. Implementing an overall preference score in combination with a numerical grading scale is a novelty in comparison to previously published studies within this field.

2.4.5 Statistical Analysis

Data were analyzed using a one-way analysis of variance (ANOVA) followed by a post-hoc Tukey’s test to examine various categorical treatment effects on continuous response variables such as pH and fermentation temperature. A one-way ANOVA followed by a Tukey’s test was used to examine the effect of treatments on LCH and flavor intensity scores. A two-way ANOVA was used to examine the effect of season on preference scores and treatment. A Simple Regression test was used to examine the relationship between pH and flavor intensity scores, as well as overall preference scores and flavor intensity. All data were analyzed using Minitab Express version 1.5.2 (State College, PA: Minitab, Inc.) at an alpha level of 0.05.

2.5 Results and discussion

2.5.1 pH

Mean starting pH of each treatment was 5.90 ± 0.4 and there was no significant difference in the mean starting pH of the cotyledon among treatments ($F_{2,18} = 0.00$, $P = 1.00$). This pH was slightly lower than pH values previously reported (6.2 – 6.9) (Afoakwa, 2016; Ali et al., 2014; Holden, 1961; Pelaez et al., 2016).

There was a significant effect of treatment on the pH of the cotyledon post fermentation ($F_{2,18} = 37.72$, $P = 0.0001$; Fig. 2-5). Mean pH values were similar between F111 (4.75) and F211 (4.74), and both were significantly different from F222 (4.37), which had the lowest mean among treatments. The results for both F111 and F211 were similar to those reported by Pelaez (2014), where mean pH values for the cotyledon post fermentation were 4.76, and higher than Hii (2006), showing mean values of 4.64. In all cases, pH values of the cotyledon decreased over the fermentation period.

Similarities between F111 and F211 were expected due to the overall consistencies in turning protocols. The significantly lower pH value of F222 could be explained by results from previous research. Wood & Lass (1985) showed that the presence of volatile and non-volatile acids, including lactic acid, citric acid, and acetic acid, contribute to a decrease in cotyledon pH. Camu (2008) noticed considerable increases in AAB populations within the fermentation after every turn. Concurrently, Wood & Lass (1985), reported high levels of acetic acid oxidation upon more frequent turnings, which resulted in beans with increased pH values. Therefore, the high frequency turning of both F111 and F211 could have resulted in an increased production of AAB, and thus high levels of acetic acid, much of which could have been lost through oxidation before being diffused into the cotyledon, resulting in a relatively high pH value. Conversely, F222 may have had a lower density of AAB and acetic acid due to infrequent turning, but could

have resulted in less acetic acid being volatilized, and was instead diffused into the cotyledon, resulting in a lower mean pH value than the other treatments.

There was an effect of treatment on mean pH values of the cotyledon post-drying ($F_{2,18} = 56.32$, $P = 0.0001$; Fig. 2-6). Means were similar between F111 (5.36) and F211 (5.42), and both were significantly different than F222 (5.03). The difference between F222 and other treatments was expected since it had a noticeably lower baseline pH due to its corresponding fermentation treatment. The overall observation that pH increased throughout drying was also made by Takrama (2006), where cotyledon pH increased from 4.2 to approximately 5.3 at the end of drying. Similarly, Hii (2006) reported the pH of dried beans to increase from 4.91 to 5.39. Mujaffar (2017) observed the increase in pH of both testa and cotyledon from 4.98, and 4.86 to 5.22, and 5.46, respectively. These results indicate a migration of water and volatile acidity from the cotyledon (Afoakwa, 2015).

2.5.2 Fermentation temperature

There were significant differences in the mean amount of time (hours) it took each fermentation treatment to reach 43.5 °C, for the top ($F_{2,15} = 6.21$, $P = 0.01$) middle ($F_{2,15} = 7.67$, $P = 0.005$) and bottom layers ($F_{2,15} = 5.01$, $P = 0.02$), as shown in Figure 2-7. F222 took a mean of 78 hours to reach this temperature, which was significantly longer than F111, which took a mean of 59 hours. The vigorous early turning of F111 may have been responsible for the rapid temperature increase over the other treatments. There may have been an increase in AAB proliferation from initial exposure to oxygen within the first 24 hours, leading to the exothermic reactions of acetic acid (Schwan & Wheals, 2004; Papalexandratou et al., 2011; Camu et al., 2008). However, there were no differences among treatments in the mean amount of time spent at or above 43.5 °C for the top layer ($F_{2,15} F = 2.68$, $P = 0.10$), or for the middle layer of the

fermentation mass ($F_{2,15} F = 2.70, P = 0.10$). Although F111 was by far the quickest in reaching the critical temperature of 43.5 °C, it was unable to maintain it any longer than the other treatments. Conversely, there were significant differences in mean times among treatments for the bottom layer of the fermentation mass ($F_{2,15} = 4.4, P = 0.03$; Figure 2-8). For the top layer, mean hours for F111, F211, and F222 were 67.83, 61.50, and 73.50, respectively. Mean hours for the middle were 66.17, 55.17, and 67.33. Mean hours for the bottom layer were 50.50, 45.17, and 66.17 respectively. F222 differed significantly from F211, and that F222 was similar to F111. The causality of these results is unclear, especially in the similarity between F222 and F111 due to the relatively drastic differences in protocols.

There were significant differences in mean maximum temperatures among treatments for the top ($F_{2,15} = 9.26, P = 0.0024$) and middle layers ($F_{2,15} = 4.81, P = 0.02$), but not for bottom layer ($F_{2,15} = 0.57, P = 0.58$; Figure 2-8), as shown in Figure 2-9. For the top layer of the fermentation, there were differences in mean maximum temperatures between F111, which had the highest mean (51.76°C) and F211, which had the lowest mean (50.19°C), whereas not for F222 (50.84°C). Similarly, means for the middle layer differed between F111 (49.54) and F211 (47.59°C), but not for F222 (48.52°C). Both F111 and F211 were similar to F222, but differed significantly from each other. These results were not expected, primarily in that F111 differed from F211 when the turning protocols are so similar. It would have been expected for F111 to be similar to F211, and different from F222. It has been previously stated that increased aeration promotes development of AAB, resulting in more acetic acid, and thus an increase in temperature. Therefore, it would be reasonable to assume that the treatments turned most frequently would result in the highest mean maximum temperatures. Conversely, the similarity between F222 and F111 could possibly be explained in that fermentations that are turned less

frequently could allow the fermentation mass to sustain higher temperatures without being interrupted and cooled down by constant turning. However, the lack of frequent exposure to oxygen could act as a barrier to temperature increase. It is therefore logical that F222 would not have the highest mean maximum temperature, and also that it would be similar to F211, in that the latter received the second to least amount of turning throughout the fermentation period.

The mean maximum bottom layer temperatures for F111, F211, and F222 were 47.04, 47.22, and 47.59°C, respectively, and were not affected by treatment. The mean was noticeably higher for F222 in comparison to the other treatments, although not significantly.

The mean amount of time (hours) to reach maximum temperatures (Figure 2-10) for F222, F111, and F211 were 132.17, 125.50, and 122.00, respectively. There were no significant differences in means between the top ($F_{2,15} = 0.35$, $P = 0.71$), middle ($F_{2,15} = 0.70$, $P = 0.51$) and bottom ($F_{2,15} = 0.59$, $P = 0.57$) among treatments. Although there are slight differences in mean values: F111 and F211 took the least amount of time in comparison, they were not significant. These results differed from studies by Camu (2008) and Guehi (2010), who both reported a decrease in time to maximum temperature in fermentations that were turned more frequently. However, the aforementioned studies employed more drastic differences between the timing of turning. For example, in both studies, the controls were not turned at all, whereas the other treatments were turned at multiple intervals throughout the fermentation period. It has often been demonstrated over the years that aeration is critical in successfully fermenting cacao (Quesnal et al., 1967; Rohan, 1963; Wood & Lass, 1985). Therefore, it would seem that the differences that were reported in these studies were conducted less from an angle of practical application, and more for the purpose of illustrating this point.

Perhaps if this study were replicated for more months, thus increasing the sample size, there would be more noticeable differences in time to maximum temperature among treatments.

2.5.3 LCH

Mean L* C* H* values are reported in Table (2-2). There were no significant differences for readings of L* ($F_{2,15} = 0.82$, $P = 0.46$), C* ($F_{2,15} = 1.59$, $P = 0.24$) or H* ($F_{2,15} = 1.42$, $P = 0.27$) among treatments. These results countered expectations in that fermentation treatments receiving a higher frequency of turning were expected to have significantly darker hue angle values due to increased oxidation of anthocyanins and polyphenols (Afoakwa, 2016). However, it could be that the turning protocols were similar enough so as to not elucidate differences among treatments. Perhaps if the treatments had a wider range of timings between turnings, more pronounced differences could have been reported.

2.5.4 Flavor intensity scores

Fresh-fruit was the only measured flavor parameter that was significantly different among treatments ($F_{3,31} = 2.84$, $P = 0.05$; Figure 2-11). F222 had the highest mean (1.27) and GS (0.28), had the lowest mean score. Although a mean of 1.27 is still relatively low on the 1-3 intensity scale, these results align with those from previous studies, which reported that beans with lower pH values often have more concentrated flavors of acidity and fruitiness (de Vos, 1956; Afoakwa, 2016), both of which were commonly used words in the flavor evaluation notes for this category (Figure 2-12). However, there was no significant effect of pH on fresh-fruit intensity scores among treatments ($F_{3,18} = 1.26$, $P = 0.31$), which contradicts the findings from these studies. These results may have been affected by the relatively small sample size of this

study ($n=7$), and because replicates were repeated over time. Perhaps if the sample size were increased for each treatment, a more noticeable relationship between pH and flavor intensity scores would emerge. Although not statistically related, it seems plausible that the lower mean pH value of F222 could have impacted the consistent recognition of fresh-fruit notes among evaluators. The increased presence of acetic acid, specifically, has been correlated to similar flavors in previous research (Campos et al., 2010), and could therefore play an important role in the aromatic quality of cacao beans.

Mean spice intensity scores were shown to have marginally significant differences among treatments ($P = 0.06$), as shown in Figure 2-13. GS (1.14) had a noticeably higher mean score than F211 (0.29), which had the lowest mean. Common words used in the flavor evaluations to describe spice included: cinnamon, allspice, clove, vanilla, and baking-spices (Figure 2-14). Although not statistically different, various flavor intensity scores such as off-flavor, have noticeably different mean scores (Figure 2-15). F111 and GS both had the highest means of 0.71, whereas F222 and F211 had relatively lower off-flavor means of 0.14 and 0.07, respectively (Figure 2-16).

2.5.5 Overall preference scores

There were no reported differences in mean overall scores among treatments between Fall 2017, and Spring 2018 ($F_{1,96} = 4.56$, $P = 0.03$), therefore data were combined between seasons. There were significant differences in mean preference scores among fermentation treatments ($F_{3,100} = 3.49$, $P = 0.02$; Figure 2-16 – 2-17). F111 (6.43), which had the highest mean (lowest preference) was significantly different from F222 (4.48), which had the lowest mean (highest preference). Treatments F211, and GS had mean scores of 4.81, and 5.24, respectively.

On the Love it / Hate it / Acceptable scale, F222 and F211 both fall within the Love it range (although F211 closely borders Acceptable), whereas GS and F111 both are firmly within the Acceptable range of the scale. These results are similar to those of previous studies, in which fermentations subjected to less frequent turning intervals resulted in beans with better flavor characteristics than those with a higher frequency of turning (Baker et al. 1994; Camu et al. 2007). However, Senenayake et al. (1997) reported that fermentations in Sri Lanka that were not turned at all produced beans with high concentrations of off-flavors compared to fermentations that were frequently turned. These results suggest that turning intervals play a critical role in determining flavor characteristics of cacao beans and chocolate, but that further research is needed to better understand the relationships between these variables. Dircks (2009) suggested that turning intervals are a critical process in fermentation, but that particular methodologies should be optimized situationally to produce desired results.

Results conflicted with the expectation that the control, GS, would score more favorably than other treatments, since it is such a well-defined standard of quality (Afoakwa, 2016). There are many factors that could have influenced the relatively low preference rating GS received.

Perhaps the specific bean samples that were sent to Dandelion Chocolate had inherent flavor defects that were not representative of Ghanaian cacao, thus skewing the results of the flavor evaluations. However, that would be an unlikely cause since the beans had no visible traces of mold contamination or any other noticeable defects. But this possibility cannot be ruled out entirely, since any amount of critical errors during post-harvest processing, such as poor fermentation methods, could have resulted in flavor deficiencies that would be visually indiscernible. There is also the prospect that the standard processing protocol used by Dandelion Chocolate was not suitable in highlighting favorable quality attributes in that particular sample.

Roasting plays an extremely important role in developing flavor characteristics that were initiated in both the fermentation and drying stages (Schwan & Wheals, 2004), and therefore the specific roasting methods that were used in this study may also have contributed to the acceptable preference ratings that GS received. It is also entirely possible that the samples were devoid of any defects and that the evaluators at Dandelion Chocolate, on average, were seeking flavor profiles other than the global standard for their individual preferences, which could account for the skewed results. It is common among craft chocolate makers to pursue flavors that are complex and nuanced, rather than plain or indistinct (Masonis et al., 2017), therefore it is understandable that, although Ghanaian cacao is a reliable standard for quality, it may not be preferable to chocolate makers that are searching for flavors beyond those standards.

Although F222 was scored most favorably among treatments, it should be noted that there were consistently high levels of mold infestation in the bottom layers of the fermentation mass, especially in the bottom corners of the box (Figure 2-18) The portion of contaminated beans, however, was not included in samples for evaluation.

Regression analysis indicated a positive relationship between off-flavor intensity and poor overall preference scores ($F_{1,26} = 5.85$, $P = 0.02$; Figure 2-19), although not among any other flavor intensities. Perhaps off flavors, which were commonly associated with words including: plastic, astringent, metallic, funky, and earthy (Figure 2-20), are more consistently identifiable flavors than other categories. The results suggest that evaluators can more likely associate off-flavors with poor preference scores, whereas flavors such as fresh-fruit or cocoa are more subjectively interpreted in terms of overall preference and quality.

2.6 Conclusion

This experiment indicates that the timing of turning can affect various fermentation parameters, including: amount of time to reach the critical temperature of 43.5°C, and the maximum temperatures attained among treatments. F111 was shown to perform most favorably in both criterion: taking the least amount of time to reach 43.5°C, and achieving the highest maximum temperature of any treatment tested. However, F111 performed relatively poorly in the organoleptic evaluations, obtaining the lowest overall preference rating, as well as a marginally higher rating for off-flavor intensity compared to other treatments. F222 and F211, although taking longer to reach 43.5°C, and attaining lower maximum temperatures than F111, did not differ significantly in sustaining critical fermentation temperatures. F222 and F211 scored more favorably for organoleptic parameters than F111 and the control, receiving the highest ratings for fresh fruit intensity, and lowest levels of off flavors. F222 was shown to have the highest overall preference score, followed by F211, both of which fell within the “Love it” range of the Love it/Acceptable, Hate it scale. These findings were similar to those of previous research where less frequent turning intervals were reported to have fewer flavor defects than those turned more frequently (Baker et al. 1994; Camu et al. 2007). F222 also had the lowest pH value among treatments, which was thought to be contributed by higher levels of acetic acid diffusion into the cotyledon due to less frequent turning. However, the consistent presence of mold contamination in F222, primarily in the bottom layers of the fermentation mass, detracts from the otherwise promising aspects of this protocol.

In summary, the F222 protocol can maximize flavor characteristics in the resulting chocolate, but should be exercised judiciously because of the likelihood of spoilage occurring in the bottom layers of the fermentation mass. Concurrently, F211 could be an effective alternative to

recommend to growers and processors, since it scored favorably in overall preference, and because of the lack of defects present in the fermentation.

2.7 Tables and Figures

Table 2-1 – Turning intervals for fermentation treatments

Sample ID	Protocol
F111	Beans are turned every 24 hrs. over a 7-day period.
F211	Beans are turned at 48 hrs. and then at 24 hr. intervals over a 7-day period.
F222	Beans are turned every 48 hrs. over a 7-day period.

Table 2-2 – Color attributes of beans among fermentation treatments

	F111	F211	F222
L*	29.61 ± 3.46	31.75 ± 3.78	31.36 ± 1.53
C*	13.59 ± 2.19	15.66 ± 2.56	13.78 ± 1.84
H*	46.52 ± 2.74	45.07 ± 2.79	47.83 ± 3.00

Values are means ± SEM, n = 3 per treatment group.

L* represents lightness, C* represents chroma, and H* represents hue on the LCH Color Space Model.

F111 was turned every 24 hours over a seven-day period. F211 was turned once after 48 hours, and then at 24-hour intervals until completion. F222 was turned every 48 hours.

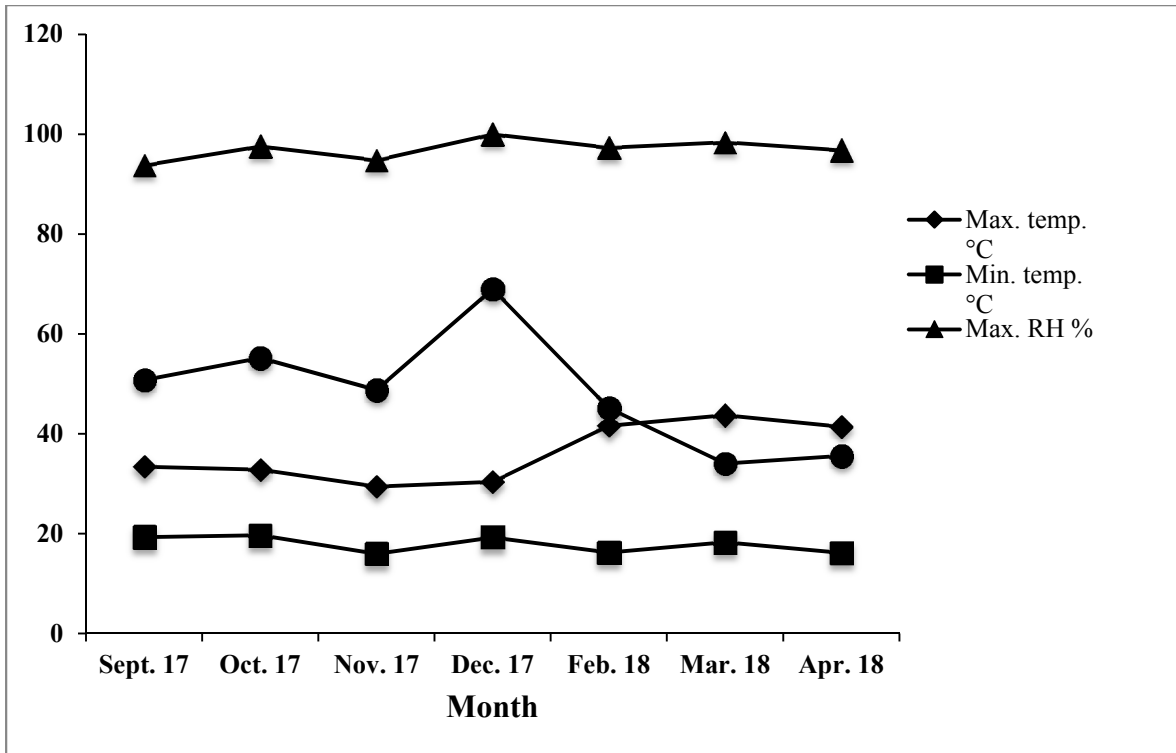


Figure 2-1 – Mean maximum and minimum values for temperature (°C) and relative humidity (%) within the fermentation greenhouse located in Pāpai'kou. Values are averaged by monthly fermentation periods.

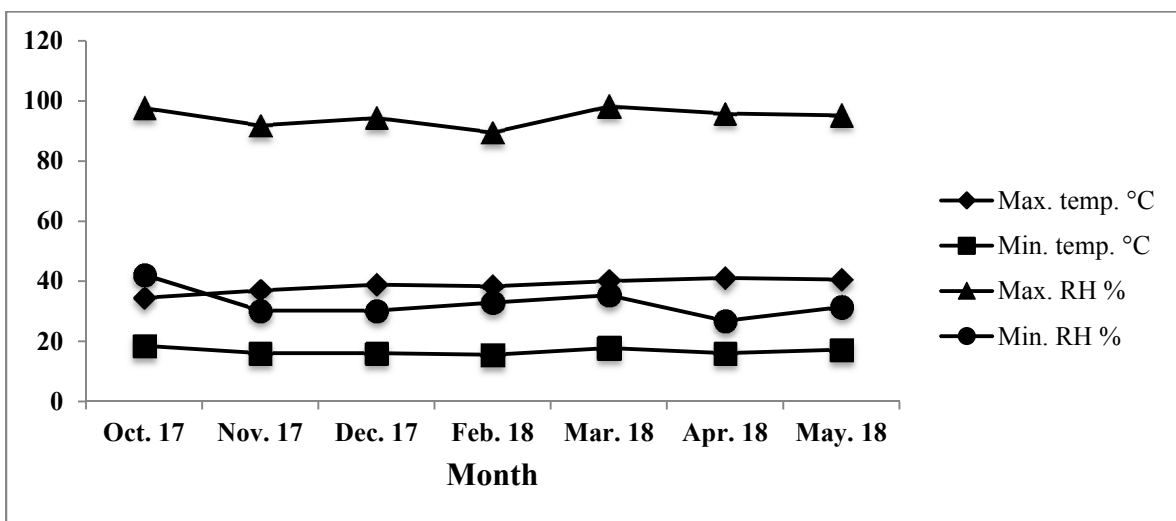


Figure 2-2 – Mean maximum and minimum values for temperature (°C) and relative humidity (%) within the drying greenhouse located in Kainaliu. Values are averaged by monthly drying periods.

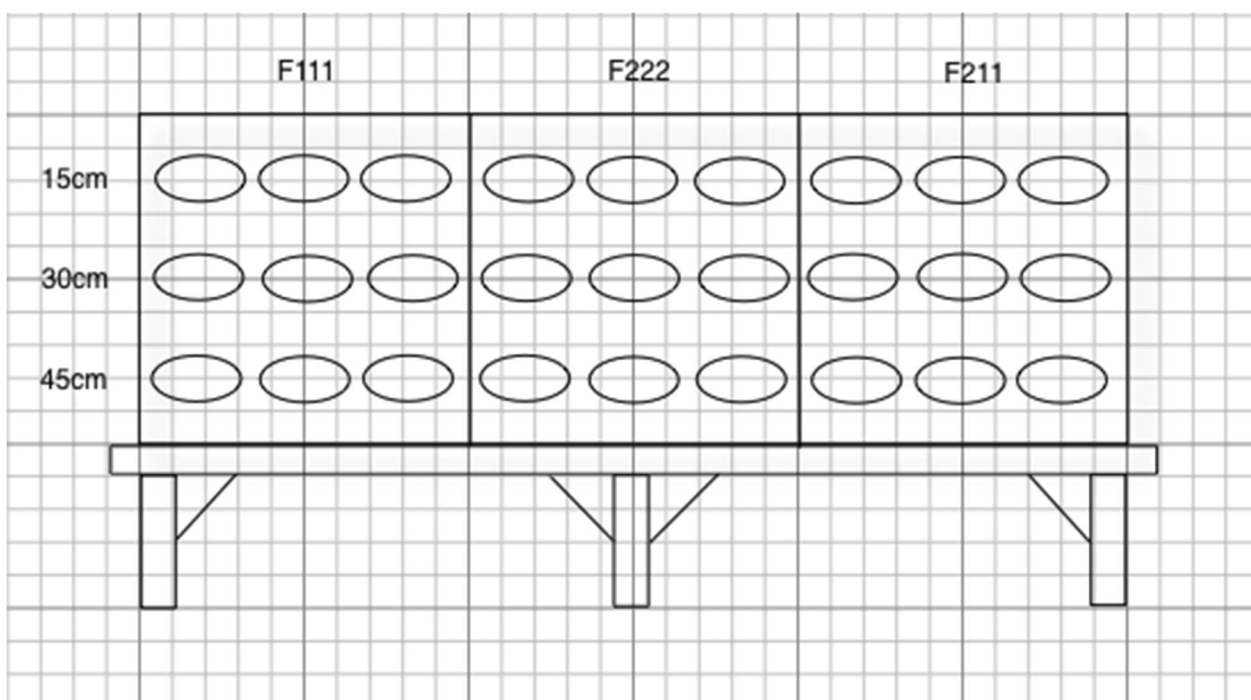


Figure 2-3 – Schematic diagram showing three separate fermentation boxes with corresponding treatments and subsample locations at various depths.

F111 is turned every 24 hours over a seven-day period, F222 is turned every 48 hours, and F211 is turned once after 48 hours, and then at 24-hour intervals until completion.



Figure 2-4 – a) Fermentation boxes with banana leaves covering the surface of the beans, b) burlap sacks covering the fermentation boxes.

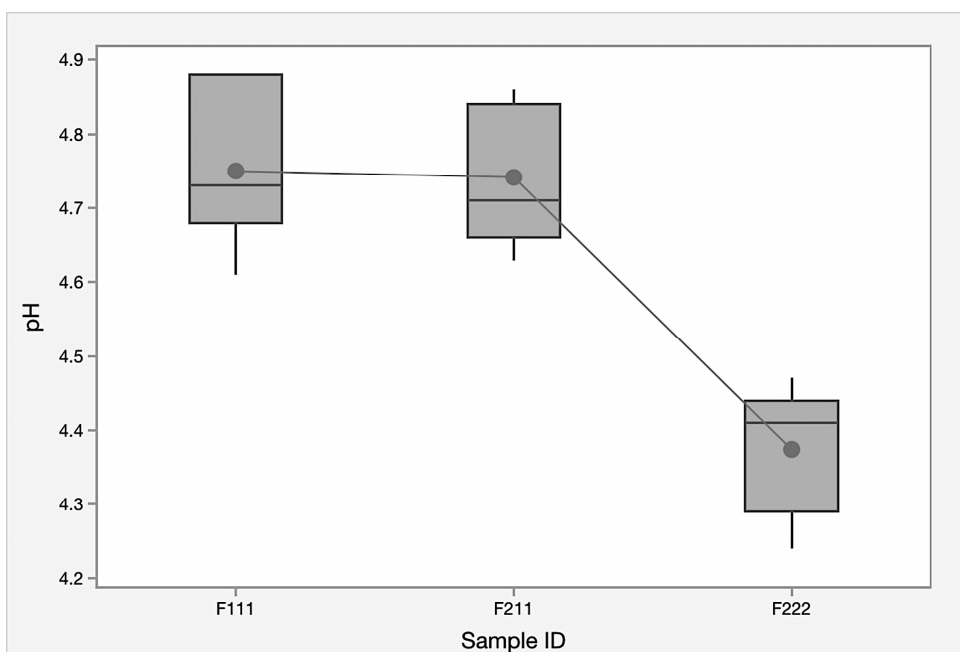


Figure 2-5 – Summary statistics for pH values of the cotyledon post-fermentation between all treatments. Circles represent means, center lines represent medians, lower and upper ranges of boxes represent first and third quartiles, respectively. Whiskers represent minimum and maximum values. F111 is turned every 24 hours over a seven-day period, F222 is turned every 48 hours, and F211 is turned once after 48 hours, and then at 24-hour intervals until completion.

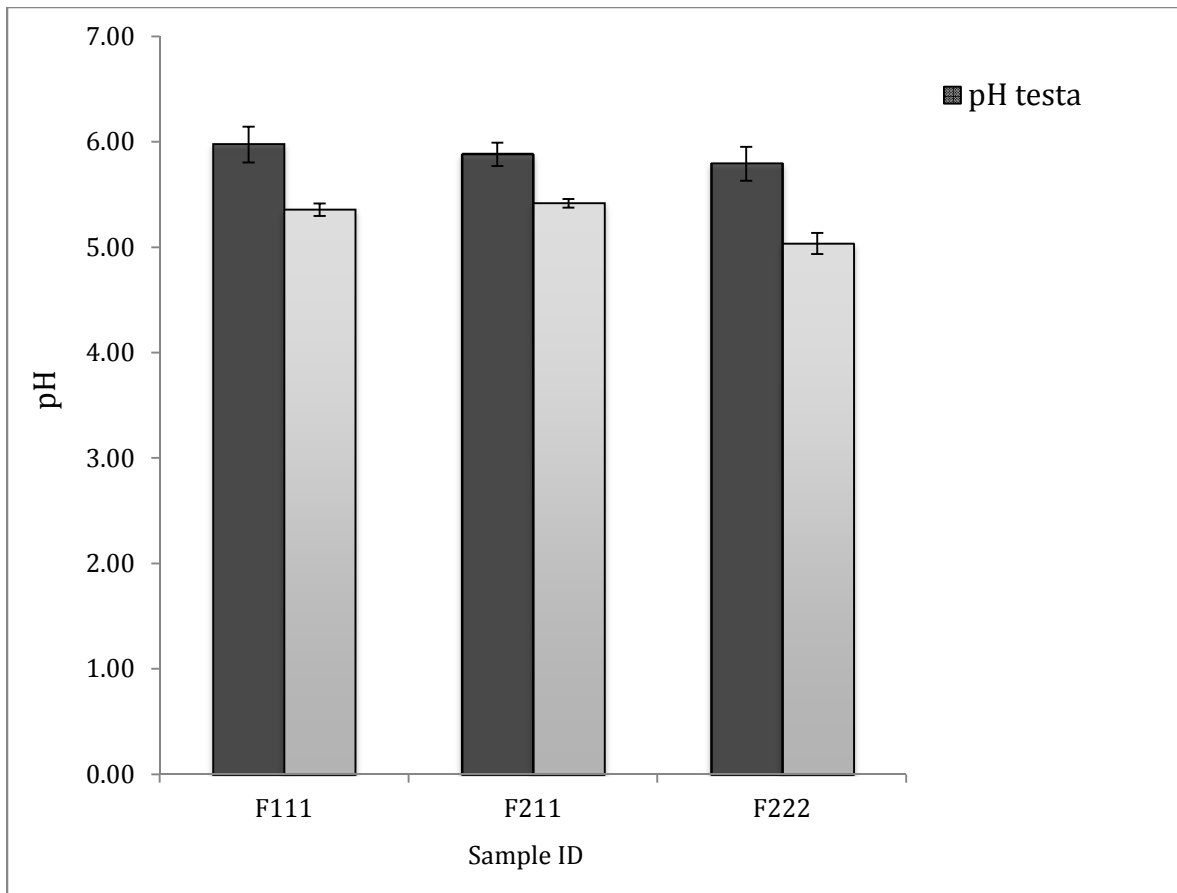


Figure 2-6 – Means and ranges for pH values of the cotyledon and testa post drying between treatments. Error bars represent Standard Deviation. F111 is turned every 24 hours over a seven-day period, F222 is turned every 48 hours, and F211 is turned once after 48 hours, and then at 24-hour intervals until completion.

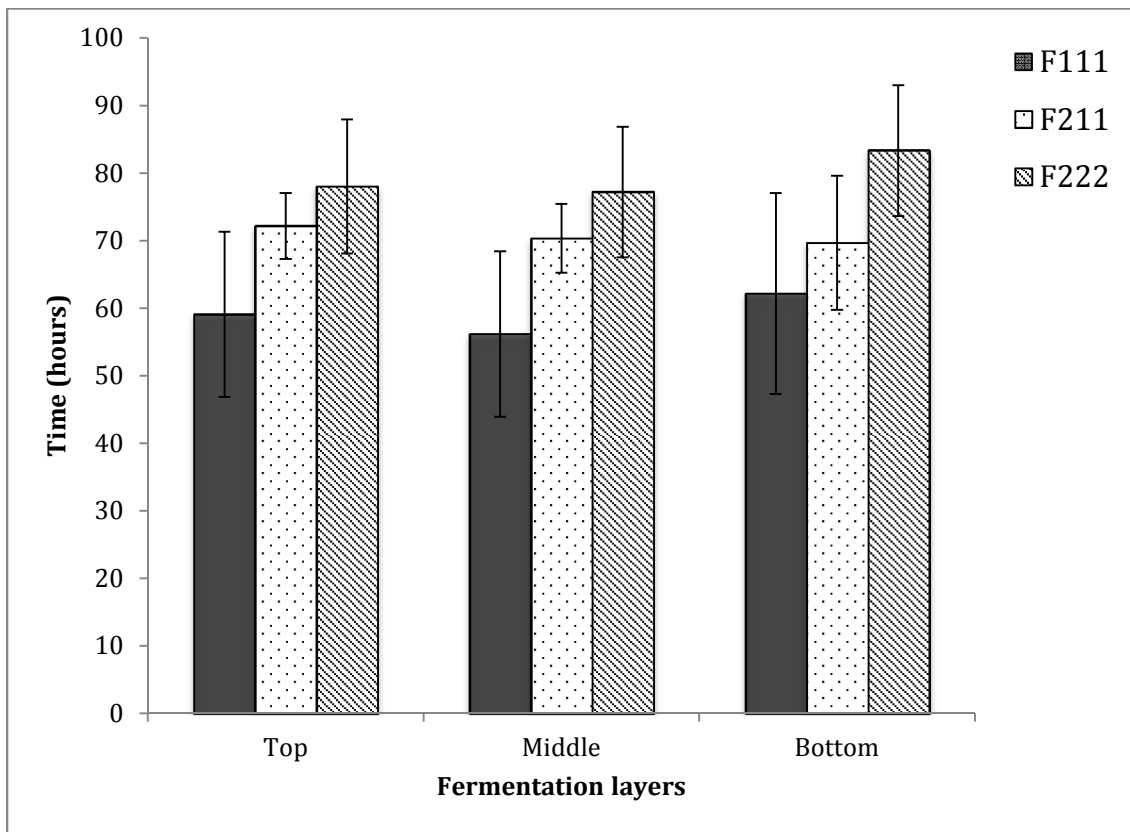


Figure 2-7 – Means and ranges for the time it took each layer of the fermentation mass to reach 43.5°C between treatments. Error bars represent Standard Deviation. F111 is turned every 24 hours over a seven-day period, F222 is turned every 48 hours, and F211 is turned once after 48 hours, and then at 24-hour intervals until completion.

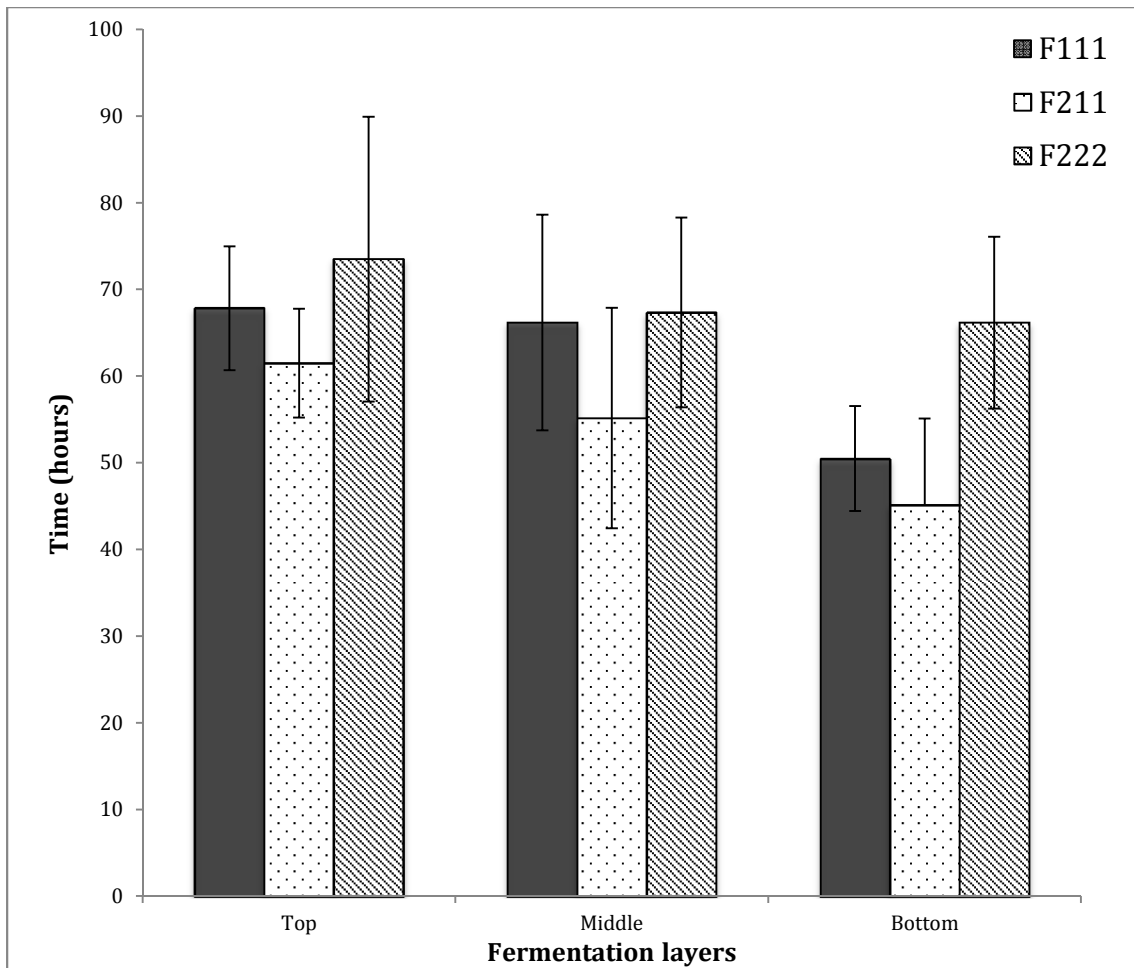


Figure 2-8 – Means and ranges for the amount of time each layer of the fermentation mass spent at or above 43.5°C between treatments. Error bars represent Standard Deviation. F111 is turned every 24 hours over a seven-day period, F222 is turned every 48 hours, and F211 is turned once after 48 hours, and then at 24-hour intervals until completion.

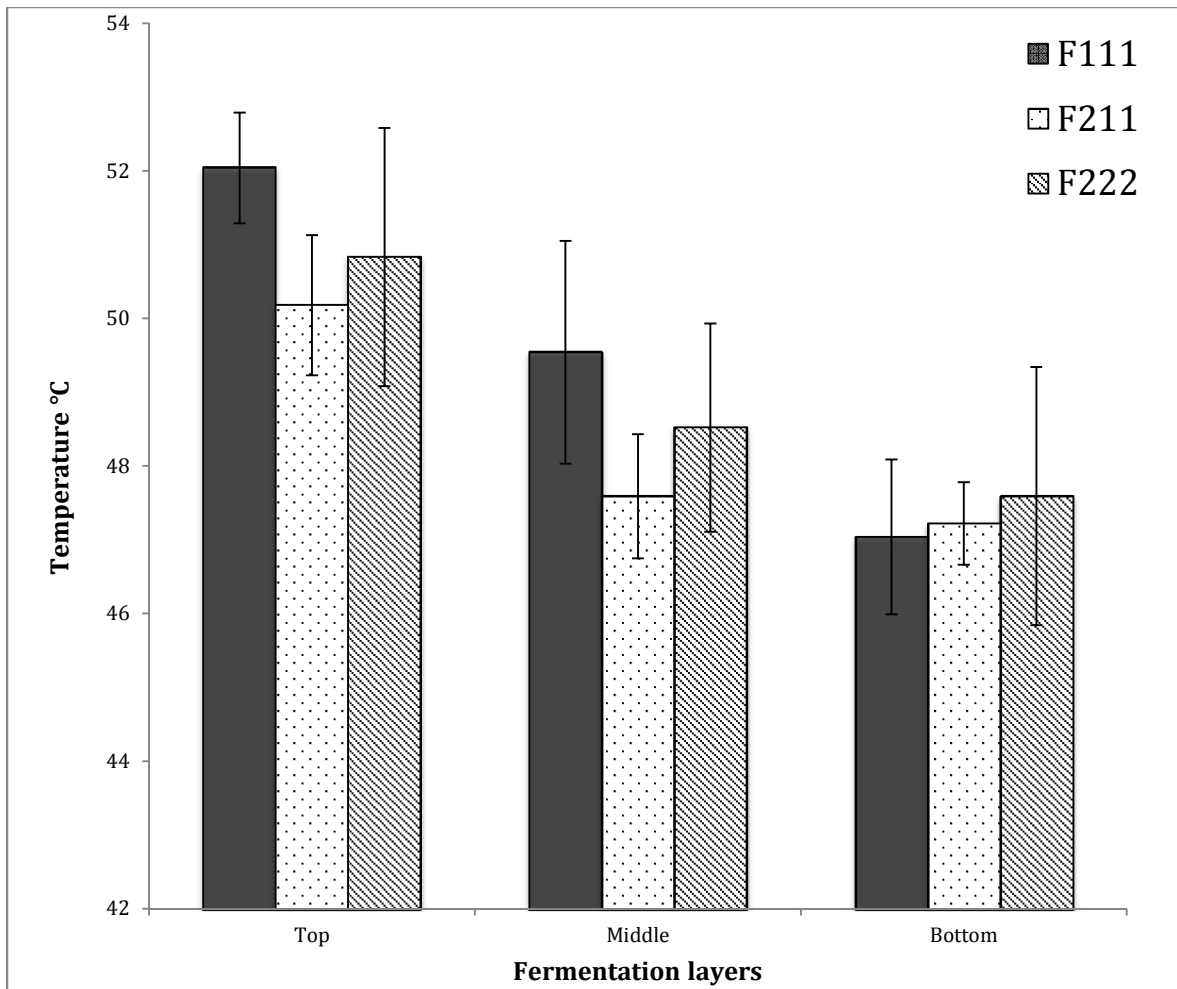


Figure 2-9 – Means and ranges for maximum temperatures (°C) reached within each layer of the fermentation mass between treatments. Error bars represent Standard Deviation. F111 is turned every 24 hours over a seven-day period, F222 is turned every 48 hours, and F211 is turned once after 48 hours, and then at 24-hour intervals until completion.

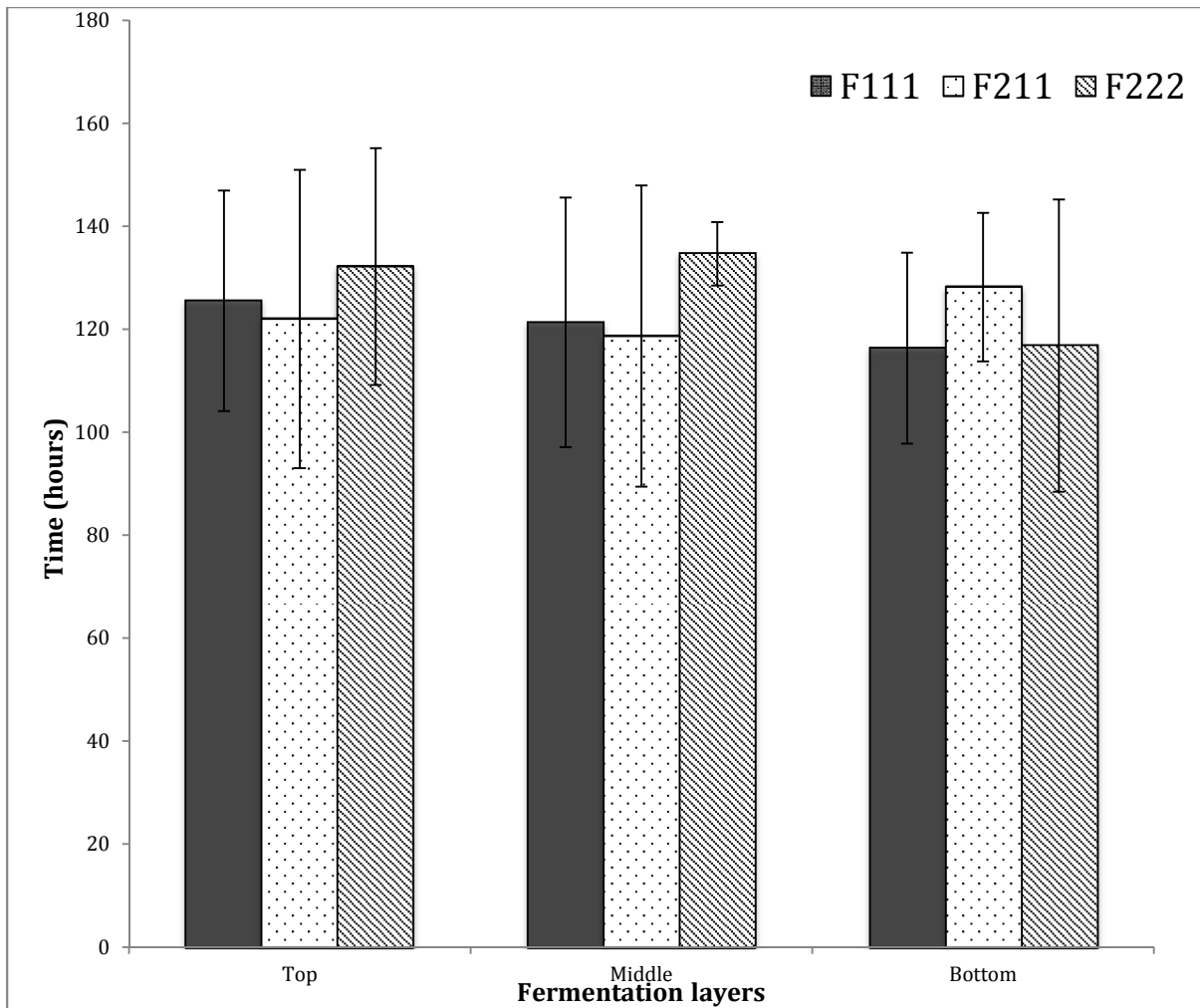


Figure 2-10 –Means and ranges for the amount of time each layer of the fermentation mass took to reach maximum temperatures (°C) between treatments. Error bars represent Standard Deviation. F111 is turned every 24 hours over a seven-day period, F222 is turned every 48 hours, and F211 is turned once after 48 hours, and then at 24-hour intervals until completion.

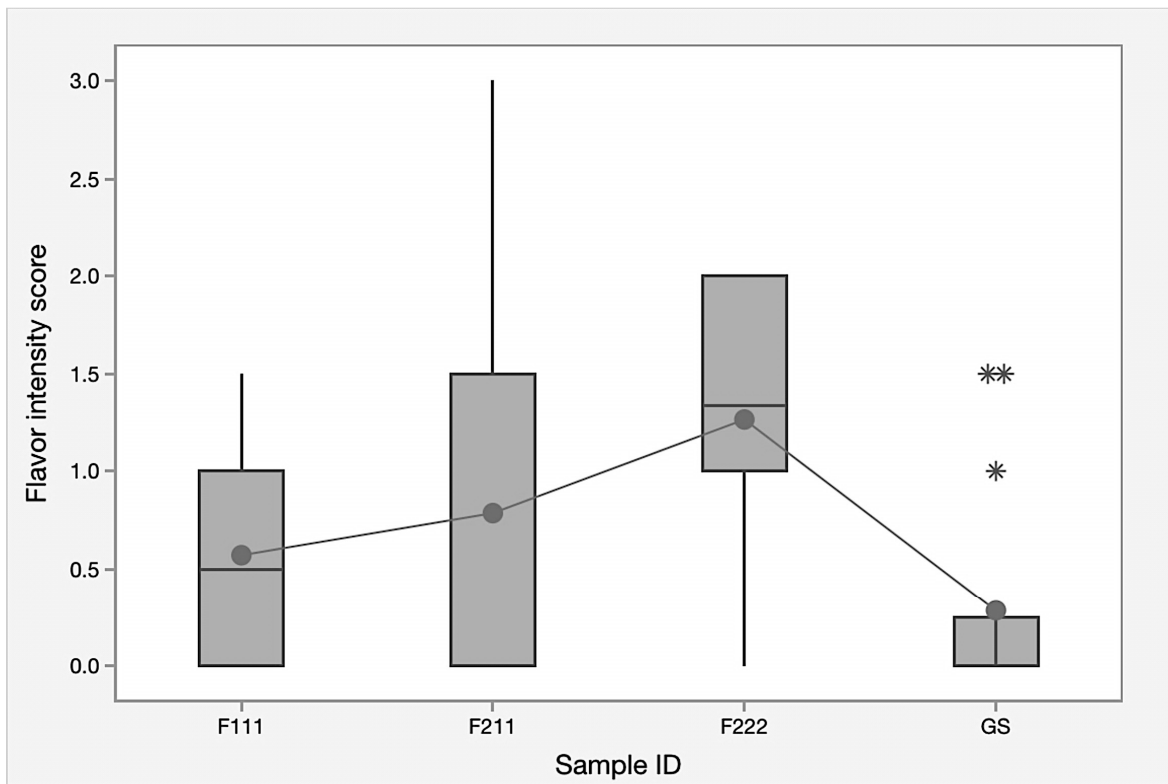


Figure 2-11 – Summary statistics for fresh fruit intensity between all treatments. Circles represent means, center lines represent medians, lower and upper ranges of boxes represent first and third quartiles, respectively. Whiskers represent minimum and maximum values. Asterisks represent outliers. Flavor intensity scale is from 0-3 (0 = not present, 1 = faint, but present, 2 = present, but not main note, 3 = dominant).

F111 is turned every 24 hours over a seven-day period, F222 is turned every 48 hours, and F211 is turned once after 48 hours, and then at 24-hour intervals until completion.

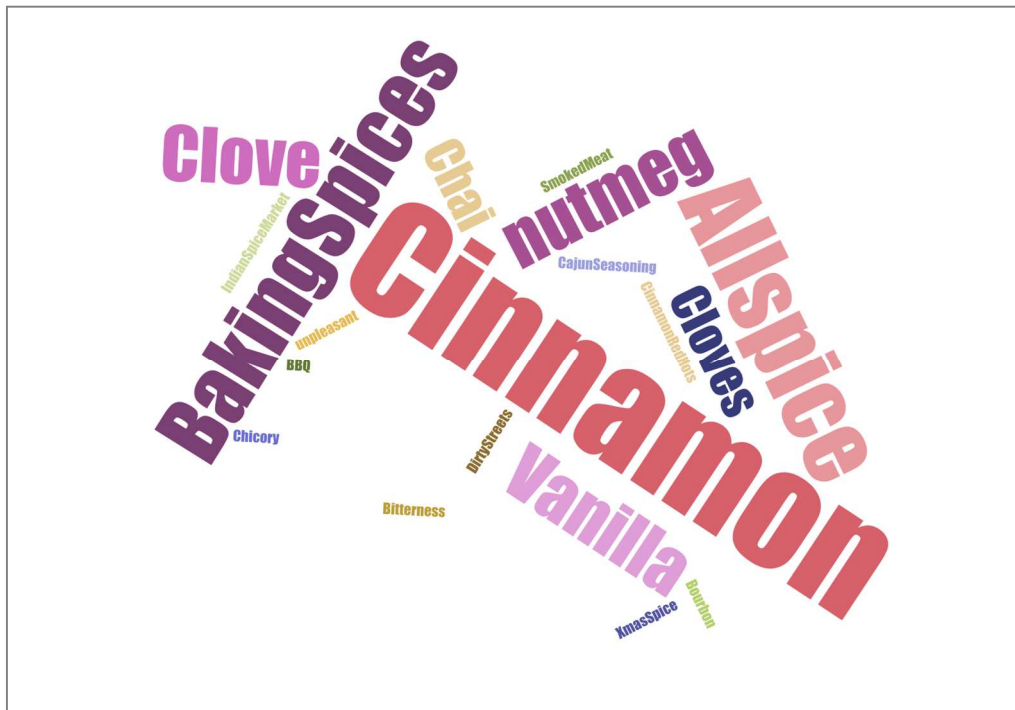


Figure 2-14 – Word Cloud representing all words used to describe samples with spice intensity. Larger words represent those used most frequently. All treatments were combined within the spice flavor category, $n = 37$.

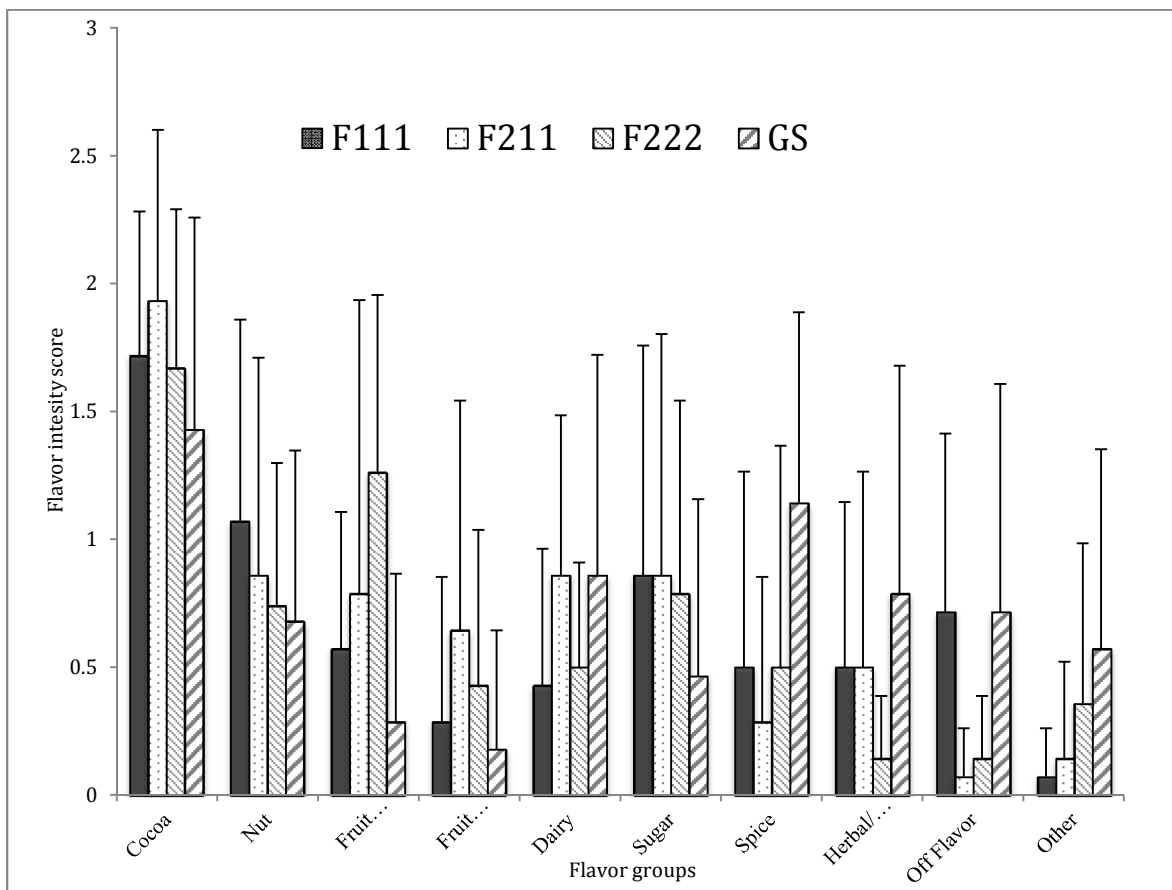


Figure 2-15 – Means and Standard Deviation for flavor intensity scores between treatments. Error bars represent Standard Deviation. Flavor intensity scale is from 0-3 (0 = not present, 1 = faint, but present, 2 = present, but not main note, 3 = dominant). F111 is turned every 24 hours over a seven-day period, F222 is turned every 48 hours, and F211 is turned once after 48 hours, and then at 24-hour intervals until completion.

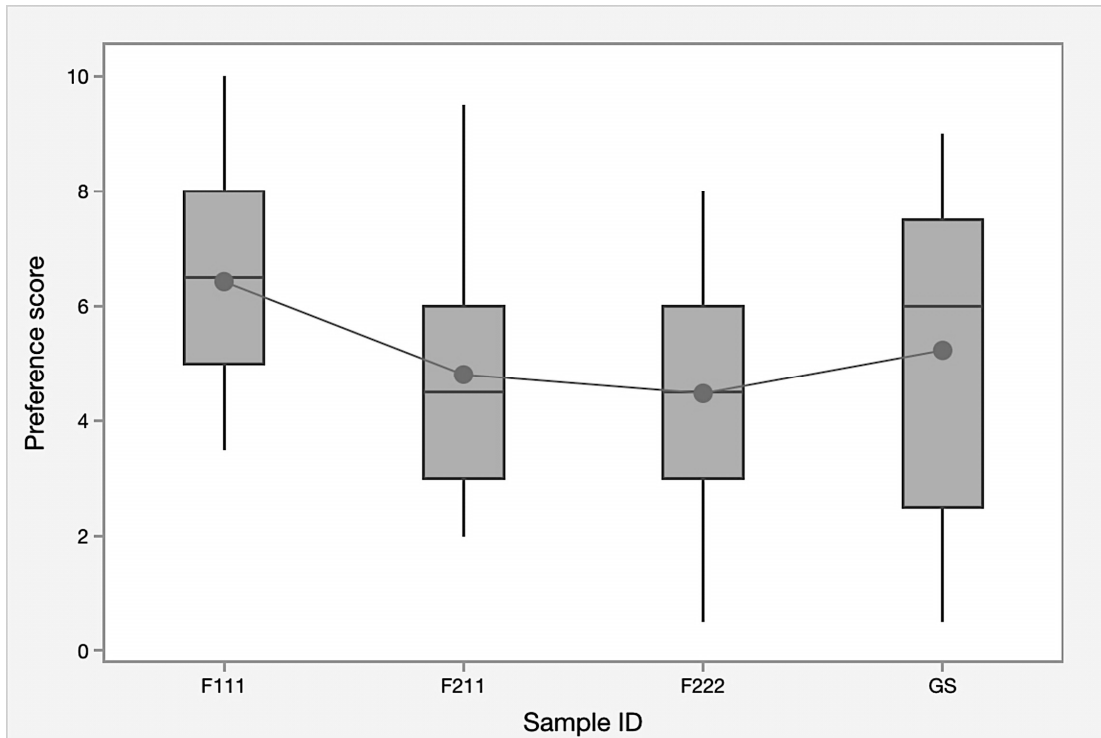


Figure 2-16 – Summary statistics for overall preference scores between all treatments. Circles represent means, center lines represent medians, lower and upper ranges of boxes represent first and third quartiles, respectively. Whiskers represent minimum and maximum values. F111 is turned every 24 hours over a seven-day period, F222 is turned every 48 hours, and F211 is turned once after 48 hours, and then at 24-hour intervals until completion.

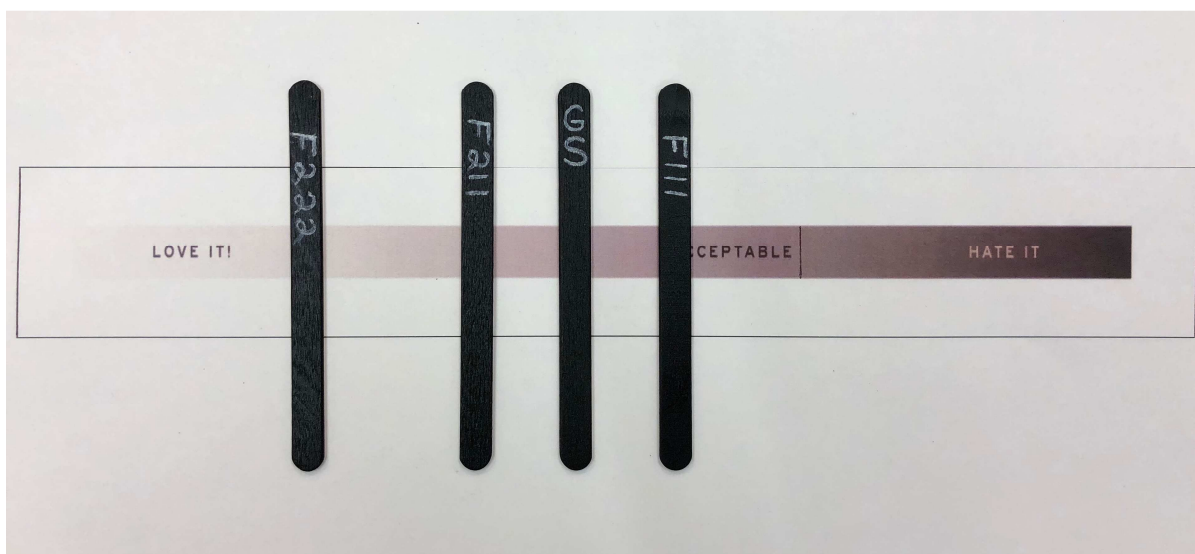


Figure 2-17 – Mean preference scores of each treatment using the Love It/Acceptable/Hate It scale.

F111 is turned every 24 hours over a seven-day period, F222 is turned every 48 hours, and F211 is turned once after 48 hours, and then at 24-hour intervals until completion.

(a)



(b)



Figure 2-18 – Various levels of mold growth in the bottom corners of a fermentation box:
a) moderate growth, b) severe growth.

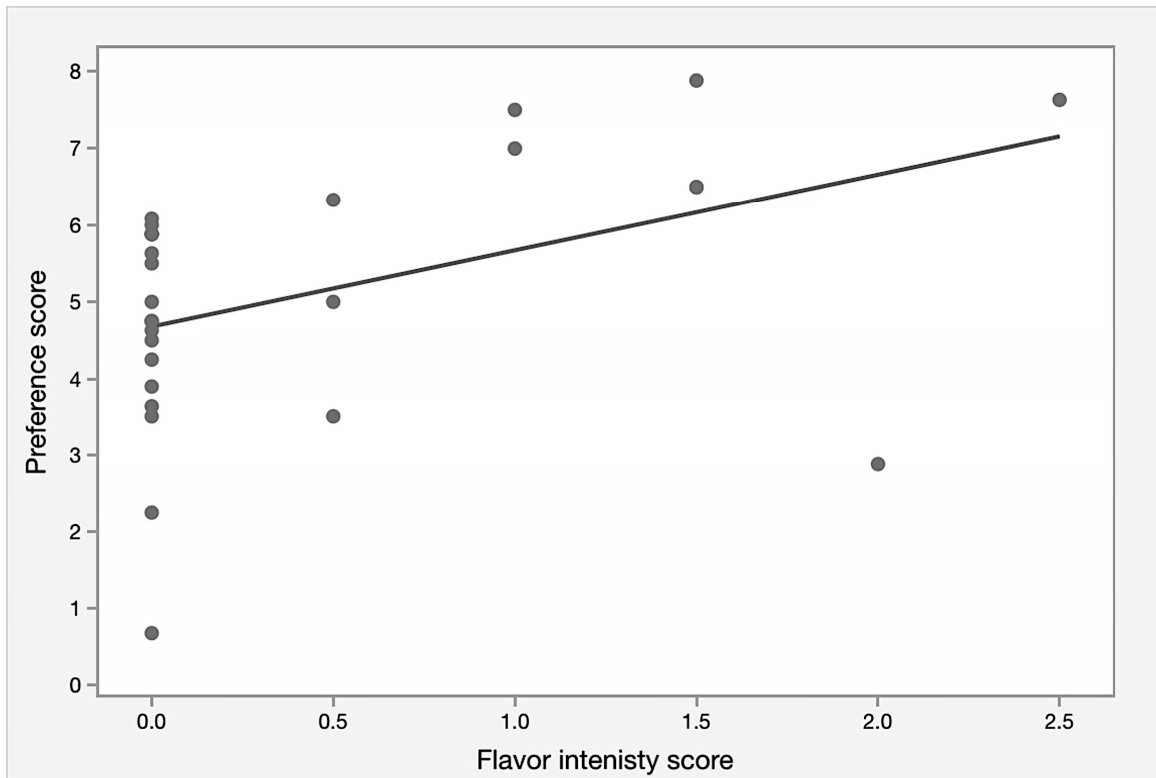


Figure 2-19 – Scatterplot with best fitting least squares regression line showing relationship between preference score and off flavor intensity.

2.8 References

- Aculey, P.C., Snitkjaer, P., Owusu, M., Bassompierre, M., Takrama, J., Norgaard, L., Petersen, M.A., Nielsen, D.S. 2010. Ghanaian cocoa fermentation characterized by spectroscopic and chromatographic methods and chemometrics, *Journal of Food Science* 1;75(6):S300-7.
- Afoakwa, E.O., J.E. Kongor, JF. Takrama, and A.S. Budu, 2013. Changes in acidification, sugars and mineral composition of cocoa pulp during fermentation of pulp pre-conditioned cocoa (*Theobroma caca*) beans. *International Food Research Journal* 20 (3): 1215-1222.
- Afoakwa, E.O. 2016. *Cocoa Production and Processing Technology*. Boca Raton, FL: Taylor & Francis Group.
- Ali, N.A., Baccus-Taylor, G.S.H., Sukha, D.A., Umaharan, P. 2014. The effect of cacao (*Theobroma cacao* L.) pulp on final flavor, Cocoa Research Centre, The University of the West Indies, St. Augustine, Trinidad and Tobago.
- Baker, D.M., K.I. Tomlins, and C. Gray, 1994. Survey of Ghanaian cocoa farmer fermentation practices and their influence on cocoa flavor. *Food Chemistry* 51:425-431.
- Camu, N., De Winter, T., Verbrugghe, M., Cleenwerck, I., Vandamme, P., Takrama, J.S., Vancanneyt, M., DeVuyst, L. 2007. Dynamics and biodiversity of populations of lactic acid bacteria and acetic acid bacteria involved in spontaneous heap fermentation of cocoa beans in Ghana. *Applied and Environmental Microbiology*, 73:1809-1824.
- Camu, N.T., K.S. De Winter, J.S. Addo, J.F. Takrama, H. Bernaert, and L. De Vuyst. 2008. Fermentation of cocoa beans: Influence of microbial activities and polyphenol concentrations on the flavor of chocolate. *Journal of the Science of Food and Agriculture* 88:2288-2297
- Dircks, H.D. 2009. Investigation into the fermentation of Australian cocoa beans and its effect on microbiology, chemistry, and flavor. PhD thesis. University of New South Wales, Sydney, Australia.
- Hart, J. H. 1900. *Cacao. A treatise on the cultivation and curing of 'cacao'* (2nd edn.) Mirror, Port-of-Spain, Trinidad.
- Hii, C.L., Abdul Rahman, R., Jinap, S. and Che Man, Y.B. 2006. Quality of cocoa beans dried using a direct solar dryer at different loadings, *Journal of the Science of Food and Agriculture*, 86 (8), 1237-1243.

Masonis, T., D'Alesandre, G., Vega, L., Gore, M. 2017. Making Chocolate: From Bean to Bar to S'more. Clarkson Potter/Publishers, New York.

Mujaffaer, S., Sukha, D. A., Ramroop, A. 2017. Comparison of the drying behavior of fermented cocoa (*Theobroma cacao* L.) beans dried in a cocoa house, greenhouse and mechanical oven. Cocoa Research Centre, The University of the West Indies, St. Augustine, Trinidad.

Papalexandratou, Z., Falony, G., Romanens, E., Jimenez, J.C., Amores, F., Daniel, H-M., De Vuyst, L. 2011. Species diversity, community dynamics, and metabolite kinetics of the microbiota associated with traditional Ecuadorian spontaneous cocoa fermentations. *Applied and Environmental Microbiology*, 77:7698-7714.

Passos, F.M.L., Silva, D.O., Lopez, A., Ferreira, C.L.L.F., and Guimares, W.V. 1984. Characterization and distribution of lactic acid bacteria from traditional cocoa bean fermentations in Bahia. *J. Food Sci.*, 49: 205-208.

Rodriguez-Campos, J., Escalona-Buendia, H.B., Contreras-Ramos, S.M., Orozco-Avila, I., Jaramillo-Flores, E., Lugo-Cervantes, E. (2012) Effect of fermentation time and drying temperature on volatile compounds in cocoa, *Food Chemistry* 132: 277-288.

Rohan, T. A. 1963. Preparation of Raw Cocoa for the Market. FAO Agric. Studies No. 60. Rome.

Schwan, R. F. and Wheals, A. E. 2004. The microbiology of cocoa fermentation and its role in chocolate quality. *Crit. Rev. Food Sci. Nutr.* 44:205-221.

Senenayake, M., Jansz, E.R., Buckle, K.A. 1997. Effect of different mixing intervals on the fermentation of cocoa beans. *Journal of the Science of Food and Agriculture*, 74: 42-48.

Sukha, D. A. 2003. Primary processing of high quality Trinidad and Tobago cocoa beans-targets, problems, options. Cocoa Research Unit, University of the West Indies, St. Augustine Trinidad.

Takrama, J.F., P.C. Aculey, and F. Aneani. 2006. Fermentation of cocoa with placenta: A scientific study. In *Proceedings of 15th International Cocoa Research Conference*; Costa Rica. Volume II, pp. 1373-1379.

Wood, G.A.R., and R.A. Lass. 1985. *Cocoa*, 4th edition. London, UK: Longman Group.

Chapter 3

THE EFFECTS OF ARTIFICIAL AND NATURAL DRYING SYSTEMS ON POSTHARVEST PARAMETERS AND QUALITY CHARACTERISTICS OF CACAO

3.1 Abstract

Drying is a crucial step in the post-harvest processing of cacao, primarily because of its effects on quality characteristics of chocolate. Sun drying is the most commonly adopted method of drying in east Hawai‘i, and can be difficult due to poor environmental conditions that correspond with peak harvest periods in this area. The development of alternative drying methods is essential to maintain a level of consistency and quality, which are necessary benchmarks for Hawai‘i-grown cacao and chocolate to succeed as a statewide industry.

This study examined the drying behavior of fermented cacao beans subjected to 12 different drying treatments which were categorized by heat source: 1) sun drying; 2) oven drying; and 3) dehumidification drying. Sun drying treatments were conducted at five locations on Hawai‘i Island. Treatments at one site (Hilo) were conducted in controlled laboratory conditions at Komohana Research & Extension Center (KREC). The other four sites (Pāpai‘kou, Pepe‘ekee, Kainaliu, and Kawaihae) represented a gradient of decreasing humidity and rainfall. Treatments at each location included both constant and intermittent cycles of drying. Moisture content was measured twice daily throughout the drying period. Color attributes, and pH were measured before and after drying. Bean samples from each treatment were sent to Dandelion Chocolate Company

for an in-depth sensory evaluation of chocolate made from each sample. Initial mean moisture content of fermented beans was $54.9 \pm 2.5\%$ wb. There were significant differences in mean drying rates between treatments, with a natural drying treatment (SDP) taking 17.9 days and only 2.9 days for a constant oven drying treatment (ODC). Mean starting pH for the testa and cotyledon was 4.54 and 4.7, respectively. A natural drying treatment (SDPC) had the highest pH value for the testa, and cotyledon, at 6.2 and 5.6 respectively, whereas an artificial treatment (DDC) had the lowest testa pH (5.5), and ODC had the lowest cotyledon pH (4.8). Results from sensory evaluations reported that an artificial intermittent treatment (ODS) had the highest rating for fresh fruit intensity (1.93), whereas the sun-dried Ghana (GS) control and DDSG had by far the lowest, of 0.29 and 0.25, respectively. Herbal/floral intensity ratings also differed between treatments, with the control having the highest rating (1.14), and a natural intermittent treatment (SDKS) having the lowest rating (0.18). Mean overall preference scores were not shown to differ between treatments.

3.2 Introduction

Hawai‘i’s cacao (*Theobroma cacao*) industry is small in comparison to other producing regions worldwide, yet is rapidly expanding. Growers throughout the state are facing many obstacles in post-harvest processing, especially relating to drying, which is a critical stage in developing flavor within the bean. Drying is particularly difficult in the eastern regions of Hawai‘i Island due to the extremely high rainfall and relative humidity that often coincide with peak harvests. Sun drying, which is the most common method of drying cacao in Hawai‘i, can take as long as three weeks under these poor environmental conditions, and beans frequently develop molds and spore forming bacteria that can negatively impact the flavor and marketability of cacao (Wood, 1975; Thompson et al., 2001; Kumi, 2005; Dahl, 2006). Growers have expressed interest in exploring alternative drying methods to increase the quality and consistency of their product.

There are two main systems of drying cacao: natural and artificial. Natural drying, or sun drying, is a system that uses a combination of solar radiation and natural airflow to dry beans, and is by far the most widely adopted method of drying (Wood & Lass, 1985). Sun drying is most commonly used because it is inexpensive and thought to be best for optimal quality, but has some definite drawbacks related to the unpredictability of drying rate (Fagunwa et al., 2009; Sukha, 2003). In regions where daytime temperatures are consistently high, resting periods are employed to control sun exposure and prevent the beans from drying too quickly (Urquhart, 1961). This method, known as intermittent drying, has been widely adopted due to its reported benefits on improving flavor characteristics of cacao (Afoakwa, 2008).

There are several methods used to simulate resting periods during drying. In the West Indies, beans are dried on lifted wooden platforms with a movable roof that can be pushed over them at set intervals to slow the rate of drying (McDonald, 1981; Bonaparte, 1995; Schwan & Fleet, 2014; Mujaffar et al., 2017). It is also a common practice to rake the drying cacao beans into large heaps at the end of each day to reduce the reabsorption of moisture during the night, when relative humidity is highest (Wood & Lass, 1985; Kumi, 2005; Amoa-Awua et al., 2007). Multiple growers in Hawai'i use this practice, especially during the wetter months when nighttime humidity can regularly reach 100%. However, instead of heaping the beans together and leaving them in a pile, they are scooped into large plastic containers and sealed with an airtight lid. Towards the final stage of drying, beans will sometimes be left in the containers all day if the ambient relative humidity is too high. During this stage (approximately 23% - 7% moisture) beans are at a lower risk of developing molds, and have a much lower chance of re-absorbing moisture by remaining in the containers until conditions are ideal.

Artificial drying is often implemented in regions where high rainfall and humidity make sun drying difficult (Asiedu, 1989). Artificial drying often involves the use of heat exchangers or direct-fired heaters to decrease the drying rate of cacao (Ghosh, 1972; Wood & Lass, 1985; Thompson et al., 2001). There are many design variations within this system, although the majority of them employ the combination of heat and airflow to dry cacao beans (Afoakwa, 2008).

The advantage of artificial drying is having more control over drying rate, which can result in a quicker turnover of marketable products. However, speed of drying should not be the only factor of concern (Sukha, 2003). There have been multiple studies on the effects of rapid artificial drying on flavor characteristics of cacao and chocolate. A study by Jinap (1994) observed that under rapid artificial drying conditions the testa, which is the semi-permeable membrane that surrounds the cotyledon, can harden (case hardening) and restrict the outward migration of moisture and volatile acidity, thus trapping it inside the cotyledon. The same study reported high levels of off flavors, including astringency and bitterness, in beans that were oven-dried at 60°C, in comparison to beans that were sun-dried. The high temperatures used in most artificial dryers can prevent critical enzymatic reactions related to flavor development, which can lead to poor quality (McDonald et al., 1981; Powell, 1982; Jinap et al., 1994; Hashim et al., 1998).

Intermittent drying protocols have been shown to reduce some of the negative implications that are often associated with rapid artificial drying (Oke & Omotayo, 2011; Mujaffar, 2017). Several studies have reported no differences in quality characteristics between natural and artificially dried beans when oven temperatures did not exceed 55-60°C (Irie et al., 2010; Oke & Omotayo., 2011). A cacao grower in Trinidad reported that the chocolate makers he sold to could not distinguish between beans that were sun-dried, and beans that were intermittently oven-dried at 57°C for two hours daily (Pers. Comm., Daniel O'Doherty, February 13, 2017). Although the studies on intermittent artificial drying contain a wide range of experimental variables, including drying temperatures, loading density, pre-treatment of beans, and exposure intervals, there seems to be a consensus on the overall potential it creates for improving consistency and quality.

Dehumidification is a novel approach to artificially drying cacao beans, and is a potential method for growers on Hawai‘i. Although it has not been critically examined in the literature, there have been several studies that tested heat pump dryers, which includes the process of dehumidification as a factor within that drying system, at temperatures of up to 56.9°C (Hii et al., 2010; Hii et al., 2012). However, as a primary source of drying, dehumidification has not been studied. There are, however, multiple anecdotal cases of growers in Hawai‘i using dehumidification to dry small amounts of cacao beans with reported success.

The purpose of this study is to evaluate the effect of alternative drying methods on post-harvest parameters and quality for Hawai‘i-grown cacao. This involves the use of various artificial drying systems, including convection ovens and dehumidifiers, as well as sun drying methods that include variations on drying location and exposure.

3.3 Materials and methods

3.3.1 Location

Drying experiments were conducted monthly between September 2017 and January 2019 at five locations on Hawai‘i island. Treatments at one location (Hilo) were conducted in temperature and humidity controlled laboratory conditions at Komohana Research & Extension Center (KREC). The other four locations represented a decreasing gradient of rainfall and relative humidity, and included: Pāpai‘kou (wettest), Pepe‘ekea, Kainaliu, and Kawaihae (driest). These locations were considered as factors within models for post-harvest parameters, including drying rate, pH, color attributes, and quality characteristics.

Drying treatments in Pāpai‘kou were conducted at Hilo Shark’s Chocolate Farm, which is situated at approximately 82 m above sea level and receives a mean annual rainfall of 3,300 mm. Treatments in Pepe‘ekea were done at Mauna Kea Cacao, LLC, which is located at about 200 m above sea level, with approximately the same rainfall as Pāpai‘kou, with cooler day and night temperatures. Treatments in Kainaliu were conducted at the Kona Research Station (KRS), located at about 365 m above sea level, with an average annual rainfall of approximately 1,500 mm. Treatments in Kawaihae took place at Hamakua Macadamia Nut Company, which is about 40 m above sea level, with an average annual rainfall of 400 mm. Data for specific on-site ambient conditions in Pepe‘ekea and Kawaihae are reported in Tables 3.1 – 3.2. Kainaliu and Pāpai‘kou are reported in Figures 3-1 – 3-2. Drying treatments in Hilo were done within controlled environments, and therefore on-site data is not provided.

3.3.2 Harvesting and cracking

Ripe cacao pods, from seedling trees of unknown parental genetics, were harvested using hand-pruners, collected in burlap sacks, hauled from the orchard and transported by truck to a central processing area at Hilo Shark's Chocolate Farm. Pods were then unloaded into a covered area and opened with machetes to extract the beans. This was done by striking the pod horizontally with the machete, and then twisting the blade so as to leverage open the pod. The split pods were then placed in a pile where the seeds were removed from the "placenta" of the pod, and placed in five-gallon plastic containers.

3.3.3 Pulp pre-conditioning

Wet seed was immediately mixed upon completion of cracking in a large perforated stainless-steel container to allow excess juice to drain from the beans prior to fermentation. This process, known as pulp pre-conditioning, has been reported to reduce acidity in the cotyledon (Afoakwa, 2016). Beans were left in the strainer for approximately two hours before being transferred into fermentation boxes. The mass was stirred every thirty minutes to promote even drainage.

3.3.4 Loading fermentation boxes

Wet seed was removed from the strainer and loaded into fermentation boxes. These boxes were constructed of untreated maple plywood, measuring 0.6 m³ per box, and 10 mm holes were drilled in the bottom of each box to facilitate drainage throughout

fermentation. Each box held 227 kg of wet seed at approximately 90% capacity, to allow for expansion of the fermentation mass. HOBO 8K Pendant Data Loggers (Onset Computer Corporation, Cape Cod, MA) were placed at the top, middle, and bottom of the fermentation mass, and were set to measure temperature at five-minute intervals throughout the fermentation period. The HOBO U12-013 Data Logger was used to measure ambient temperature (°C), and relative humidity (%) within the greenhouse. Beans were subjected to a standard turning protocol used in Hawaii, which involved turning the fermentation once after 48 hours and then at 24-hour intervals every day thereafter over a seven-day period. To adhere to common fermentation practices, fresh banana leaves were used to cover the surface of the fermentation mass, followed by a thick, but loosely assembled layer of burlap sacks to provide added insulation (Sukha, 2003; Wood & Lass, 1985). The fermentation was turned from one box to another using a plastic grain scoop. On approximately the fourth day of fermentation, banana leaves were placed along the bottom of each fermentation box before the beans were turned. This was done to cover the drainage holes, since most of the moisture in the mucilage surrounding the beans had drained during the fermentation process. This practice also helps to retain moisture and heat in the bottom layer of the fermentation mass, reducing the likelihood of molds and spore forming bacteria infecting the fermentation (Pers. Comm., Daniel O'Doherty, July 3, 2017).

3.3.5 Implementing drying protocols

Upon completion of fermentation, approximately 20 kg of beans were removed from the box and evenly divided onto eight separate drying screens, so that each screen held 2.5 kg

of beans. Screens measured 50.8 cm x 40.6 cm, and were made using food grade plastic mesh with an untreated pinewood frame. The beans were spread on the screens at a depth of 5 cm. Each screen was randomly assigned to a drying treatment and transported to their respective locations.

3.3.6 Drying treatments

Treatments included 12 distinct drying protocols (Table 3.3) which were categorized by heat source: 1) sun drying; 2) oven drying; and 3) dehumidification drying. Location, exposure interval and sample size, were additional variables among treatments.

Sun drying treatments included: Sun Dry Pāpai‘kou (SDP), Sun Dry Pepe‘ekea Constant (SDPC), Sun Dry Pepe‘ekea Standard (SDPS), Sun Dry Kainaliu Constant (SDKC), Sun Dry Kainaliu Standard (SDKS), Sun Dry Kawaihae Constant (SDKWC), and Sun Dry Kawaihae Standard (SDKWS). Oven drying treatments included: Oven Dry Constant (ODC), Oven Dry Standard (ODS), and Oven Dry Trinidad (ODT). Dehumidification drying treatments included: Dehumidification Dry Constant (DDC), and Dehumidification Dry Step-Down (DDSD).

Treatments: SDPS, SDKWS, DDC, DDSD and ODT, were novel intermittent drying protocols that were recommended by growers and collaborators. Treatments SDP, SDKS, and ODS, received the same industry accepted standard intermittent-drying protocol, which involved three hours of heat exposure the first day (9am-12am), four hours the second day (9am-1pm), six hours the third day (9am-3pm), and eight hours every day thereafter (9am-5pm) until the beans reached the industry accepted standard of 7-7.5 % moisture (Afoakwa, 2008).

Samples from all treatments were stirred vigorously by hand every 30 minutes (or as frequently as possible) during the first day of drying (from approximately 9am to 5pm), every hour during the second day, every three hours the third day, and three times per day on each of the subsequent days until the beans were dry. Stirring was done for multiple reasons: 1) to promote uniformity in drying among samples; 2) to break apart aggregates that may have formed during fermentation (Hart, 1900); and 3) to polish the beans by rubbing them vigorously against the plastic mesh of the screens.

Upon completion of drying, samples were removed from their trays and placed in separate mesh insect-proof bags and stored at ambient temperature and relative humidity within a lab at KREC for at least six weeks before being blended, packed, and submitted for organoleptic evaluations (Pers. Comm., Ed Seguire, May 20, 2017).

3.3.7 Environmental data collection

An Onset S-WSB-M003 Wind Speed Smart Sensor was used to calculate wind speed (m/s) at thirty-second intervals at each drying location. A HOBO U12-013 Data Logger was used to measure ambient temperature (°C) relative humidity (%), and light intensity (l m) at each location. For sun-drying treatments, sensors were placed adjacent to the drying screens, and for both the oven-drying and dehumidification-drying, sensors were placed within their respective drying unit.

3.3.8 Moisture data

Initial moisture readings of the beans were taken immediately after the completion of fermentation, as well as twice daily from each treatment throughout the drying cycle.

Moisture content was calculated using the gravimetric method: 100 g samples were placed on mesh screens within a scientific convection oven at 110°C until there was no further loss in mass (approximately 48 hours). The following formula was used to calculate moisture content for each sample.

Moisture % = (fresh weight – dry weight) / dry weight).

3.3.9 pH data

pH of cotyledon and testa were taken before and after drying throughout all treatments. Samples (10 g) of beans were manually husked to remove the testa from the cotyledon, and then ground separately using a mortar and pestle. Samples were then homogenized for 30 seconds in 100 ml of distilled water. A 25 ml aliquot was pipetted into a beaker and the pH was measured using an Oakton pH 150 meter (OAKTON Instruments, Vernon Hills, IL).

3.4.1 Color data

Color attributes of the beans were measured post drying among all treatments, using a Minolta Chroma Meter CR-300 (Konica Minolta, Inc.). L*C*H* represent three axes on the LCH Color Space Model (Figure 3-3). The L* axis is vertical and represents Lightness: the range from absolute black to absolute white. The C* axis represents Chroma (saturation), which ranges from completely unsaturated (i.e. natural grey, white, or black) to high saturation. H* is the circular axis on the model, and represents hue angle, ranging from 0° (red) through 90° (yellow), 180° (green), and 270° (blue). Triplicate L*, C*, and H* readings were measured for each treatment.

3.4.2 Blending

After samples from each treatment had cured in breathable plastic mesh sacks for at least six weeks, they were combined within their respective treatment samples for each season (Fall 2017, Spring 2018, and Fall 2018). This allowed for a single representative sample for each treatment per season. Blending was done for two main reasons: 1) to reduce the number of samples for evaluators to process, and 2) to mimic farming procedures where beans from different lots are blended according to quality to promote uniformity in marketable bean shipments. The mixture was then sorted to remove germinated, broken, or under-developed beans. A 1,600 g sample was then taken from the mixture and sealed in a one-gallon Ziploc® bag (S.C. Johnson & Son, Inc.). This process was repeated for beans among each treatment.

3.4.3 List of samples sent for evaluation

Samples were packaged and sent to Dandelion Chocolate Company in San Francisco CA. for evaluation. The sample list included the following drying treatments: SDP, SDPC, SDPS, SDKC, SDKS, SDKWC, SDKWS, ODC, ODS, ODT, DDC, DDS, as well as a sample of Ghana-grown beans (GS) to act as a control for quality. Ghanaian cacao is the standard by which all cacao is measured, and is commonly used in organoleptic evaluations as a reference point in identifying key flavor components within samples (Afoakwa, 2016). The first set of samples, which represented the Fall 2017 harvest season (Sept. – Dec.), were sent in February 2018, the second set, which represented the

Spring 2018 season (Jan. – May), were sent in June of 2018, and the third set, which represented the Fall 2018 harvest (Oct. – Dec.), was sent in April 2019.

3.5 Organoleptic evaluations by Dandelion Chocolate

3.5.1 Processing samples into chocolate

Samples were processed according to a standard protocol developed by Dandelion Chocolate Company: Approximately 1600 g of cacao beans were roasted in a Behmor 1600 coffee roaster (Behmor Inc. Incline Village, NV) at 120°C for 17.5 minutes, with a 13-minute cool down period. After the beans were cracked and winnowed, 1 kg of 70% chocolate was made, consisting of 700 g of cacao nibs and 300 g of unrefined cane sugar. The chocolate was ground and conched for 18-20 hours using a mini *mélanger*, reducing the particle size to approximately 25 microns. Untempered chocolate was then poured into 10 mm square molds where they were stored until the time of evaluation.

3.5.2 Sample preparation and evaluation methods

There was a total of seven evaluators from Dandelion Chocolate. They were essentially untrained and un-calibrated, but were chosen specifically for two reasons: 1) They were well practiced tasters: each of them had constant exposure to cacao beans and chocolate from a wide range of origins and quality. 2) They were thoughtful and reliable tasters that were capable of committing to the evaluation schedule, and taking their role seriously. Each of the seven evaluators completed two replicates of each sample per season (Fall 2017, and Spring 2018) over an eight-month period. All samples were blinded with two unique 3-digit blind codes that related to the replication number.

The order in which samples were tasted was randomized, however replicate samples were intentionally spread out to ensure that evaluators would not taste the same samples back to back. Evaluators used the GoCanvas application (2018 Canvas Solutions, Inc.) to directly input data, rather than having to transfer outputs from a handwritten sheet. This was done to promote efficiency during evaluations, and to minimize the possibility of data entry error.

3.5.3 Intensity scaling methods

Instead of relying on each individual evaluator's complex aromatic memories to define the samples, the decision was made to define a much smaller number of categorical aromatic groups: Cocoa, nut, fresh-fruit, dried-fruit, dairy, sugar, spice, herbal/floral, other, and off-flavor intensities. Karen Cogan, Flavor Manager at Dandelion Chocolate, felt it would be easier to find more of a consensus among evaluators when confined to larger themes. For example, in using this method, if all seven evaluators reported the chocolate samples tasting like "fresh fruit", as opposed to a more highly specific flavor descriptor such as "lemon cake" then it could be concluded more reliably that the evaluator indeed meant "fresh fruit" rather than the potential "cake" or "lemon", either of which being plausible deductions from "lemon cake".

Due to the untrained nature of the panel, a relatively basic intensity scale was chosen:

0 - Not Present

1- Faint, but present

2 - Present, but not main note

3 - Dominant note

This was designed to force evaluators into making a concise decision on the intensity of detected flavors. The more commonly used 0-10 scale leaves more room for subtle differences in flavor between samples, but also creates a wider space for error when score results are combined among multiple evaluators. A Word Cloud generator was implemented to display common words or phrases used in each flavor intensity category. This was done to better visualize the data among evaluators.

3.5.4 Preference scaling method

The Love it/Acceptable/Hate it Scale (Figure 3-4) was developed to offer a sense of actual preference between samples. For example, when an evaluator scores a particular sample with a “3” for cocoa intensity and a “1” for fresh-fruit, a clear differentiation between main flavor groups is established, but a sense of overall quality is relatively untouched. This categorical scale gives the evaluator a chance to state if they actually like the sample they just tasted, or not. Including this categorical method of evaluation in the study is important because it represents a facet of the decision making process that industry professionals use when purchasing cacao from various origins. Implementing an overall preference score in combination with a numerical grading scale is a novelty in comparison to previously published studies within this field.

3.5.5 Statistical analysis

Data were analyzed using a one-way analysis of variance (ANOVA) followed by a post-hoc Tukey's test to examine various categorical treatment effects on continuous response variables such as pH, and moisture content. A one-way ANOVA followed by a Tukey's test was also used to examine the effect of treatments on L*C*H* and flavor intensity scores. A two-way ANOVA was used to examine the effect of season on flavor intensity, and preference scores among treatments. A Simple Regression test was used to examine the relationship between pH and flavor intensity scores. A Two Sample t - Test was used to examine mean scores of individual treatments between seasons. All data were analyzed using Minitab Express version 1.5.2 (State College, PA: Minitab, Inc) at an alpha level of 0.05.

3.6 Results and discussion

3.6.1 Drying rates

The mean starting moisture content for beans was $54.9 \pm 2.5\%$. These results are similar to those reported by Fagunwa et al., 2009 and Mujaffar et al., 2017, which had starting means of 53.4%, and 50.9, respectively.

There were significant differences in mean drying rates (days) between treatments ($F_{11,60} = 21.13$, $P = <0.0001$; Figure 3-5). Mean rates for ODC (2.88) and DDC (3.17) were the lowest, whereas SDP (17.9) had a significantly higher mean in comparison to all other treatments. These results corroborate previous observations that natural sun-drying systems generally take longer than a non-solar (Wood and Lass, 1985; Fagunwa et al., 2009; Mujaffar et al., 2017; de Vos, 1956; Afoakwa, 2016). The exceptions were

SDKWC (3.33), which dried quicker than DDS, ODS, and ODT, as well as SDKWS, which dried quicker than ODS and ODT, although not significantly. SDKWC had a significantly faster drying time than any other sun drying treatment, but was only replicated during Fall 2018, and therefore had a much smaller sample size ($n=3$) than the other treatments.

SDPC and SDPS both had lower mean drying times than the control SDP. This could have been attributed to the open-air drying conditions of both treatments compared to the higher humidity and reduced airflow of the control. Previous studies have shown that drying rate is greatly diminished when airflow is restricted, especially during the early stages of drying (Bravo & McGraw, 1974; McDonald et al., 1981; Thien & Yap, 1994; Fagunwa et al., 2009). These results suggest that SDPS and SDPC could be possible alternatives to the drying area of SDP to reduce drying time, however, the smaller sample size of these treatments ($n=3$) compared to SDPC ($n=11$), limits the applicability of these results.

During the second falling-rate stage of drying ($\approx 23\% - 7\%$), moisture levels of SDPS would often remain constant overnight, whereas SDPC would rise in moisture content. The protocol of sealing the beans in plastic containers at night, when RH is highest, possibly limited the re-absorption of moisture, whereas SDPC, which was left on the drying screens constantly, had more interstitial moisture to evaporate, and thus slowed the rate of drying (Fagunwa, 2009). In contrast, the average nighttime RH was low enough in Kawaihae that SDKWC maintained constant moisture, and in some cases continued to dry, whereas SDKWS, which was heaped and covered nightly would re-absorb moisture, especially during the final stages of drying.

3.6.2 pH

Mean starting pH for the cotyledon and testa were 4.70 and 4.53, respectively. These results are similar to previous studies. Hii et al., 2006, reported starting pH values of the cotyledon at 4.64, whereas Mujaffar et al., 2017, saw values of 4.98, and 4.86 for the cotyledon and testa.

There were significant differences in mean end pH for both the cotyledon ($F_{11,60} = 6.89$, $P = <0.0001$) and testa ($F_{11,60} = 5.52$, $P = <0.000$) among treatments (Figure 3-6). SDPC had the highest mean pH for the cotyledon (5.60) and testa (6.19), and was significantly different from all treatments except SDP (5.36) and SDKS (5.19). ODC had the lowest mean cotyledon pH (4.81), and was significantly different from SDPC, SDP, and SDKS. DDC had the lowest mean testa pH (5.46) among treatments, and differed significantly from SDPC (6.19), SDKS (6.10), SDKC (6.06), SDP (6.05), and DDSD (6.04). These reports were similar to those found by Jinap et al., 1994, where rapidly dried beans resulted in increased levels of acetic acid retention, which lowered pH. Regression analysis showed a positive linear relationship between drying time and pH, for both the cotyledon ($F_{1,82} = 32.78$, $P = <0.0001$; Figure 3-7), and the testa ($F_{1,70} = 23.23$, $P = <0.0001$; Figure 3-8) when means were combined among treatments. Overall, Figure 3-6 shows that pH of both the cotyledon and testa increased after drying. This observation was also made by Takrama and others (2006) where cotyledon pH increased from 4.2 to approximately 5.3 at the end of drying. Similarly, Hii and others (2006) reported the pH of dried beans to increase from 4.91 to 5.39. Another study (Mujaffar et al., 2017) demonstrated an increase in pH of both testa and cotyledon from 4.98 to 5.22,

and 4.86 to 5.46, respectively, for different drying systems. These results were explained by a migration of water and volatile acidity from the cotyledon (Afoakwa, 2016).

3.6.3 L*C*H*

There were no differences among treatments for mean L* readings ($F_{11,15} = 1.04$, $P = 0.46$). Treatments SDPC and SDPS had higher mean values than other treatments, but the differences were not significant, likely partially due to the small sample sizes ($n=3$). Similarly, Mujaffar and others (2017) reported that drying method did not affect bean lightness. However, a 1997 study by Bonaparte and colleagues reported higher L* values for beans that were sun-dried in comparison to beans that were dried using a direct type solar drier.

There were differences in C* ($F_{11,15} = 4.21$, $P = 0.01$), and H* values ($F_{11,15} = 3.65$, $P = 0.01$) between treatments (Table 3-4). SDKWC had the highest mean C* value, and was significantly different from SDKC, SDP, DDSO, ODS, SDKS, ODT, and ODC. SDPC and DDSO were shown to have the highest H* values among treatments, and were significantly different from ODC, which had the lowest mean. Similar results were reported by Hii and others (2009), where H* values were significantly higher for sun-dried beans over oven-dried beans. This could be attributed to oxidative reactions of anthocyanins and polyphenols in beans with increased exposure to environmental factors (Afoakwa, 2016).

3.6.4 Flavor – intensity scores

There was no effect of season on individual flavor intensity scores; therefore, the data from Fall 2017, and Spring 2018, were combined. The only flavor groups that were affected by treatment were fresh-fruit ($F_{8,121} = 6.95$, $P = <0.000$; Figure 3-9 to 3-10), and herbal/floral ($F_{8,121} = 3.14$, $P = 0.002$; Figure 3.9 to 3.11). ODS (1.93) had the highest mean rating for fresh-fruit intensity, whereas GS (0.29) and DDS (0.25) had by far the lowest mean ratings. The oven-dried treatments had higher mean ratings than both the sun-dried, and dehumidification dried treatments, but the differences were not statistically significant (Figure 3.10). GS (1.14) had the highest mean rating for herbal/floral intensity, whereas SDKS (0.18) had the lowest rating (Figure 3.11).

Regression analysis showed no relationship between end pH of either the cotyledon ($F_{1,19} = 1.91$, $P = 0.18$) or testa ($F_{1,19} = 0.76$, $P = 0.40$) on individual flavor intensity scores among treatments. These results countered expectations in that low pH is often correlated to fruity and acidic flavors in cacao beans and chocolate (De Vos, 1956; Howat et al., 1957; Jinap et al., 1994; Rodriguez-Campos et al., 2010). The scatterplot showed extreme variation among evaluators, and no visible trend between flavor intensity and acidity of the bean.

3.6.5 Preference scores

There were no significant effects of season on overall preference scores among treatments ($F_{1,223} = 2.35$, $P = 0.13$), therefore data from Fall 2017, and Spring 2018 were combined. This may demonstrate a certain level of overall consistency among evaluators, regarding their ability to give similar scores to samples between seasons.

Despite differences in flavor intensity ratings, there were no differences in mean overall preference scores among treatments ($F_{8,232} = 1.42$, $P = 0.19$; Figure 3-12). The high degree of variability among individual evaluators may have contributed to a lack of significant differences between treatments. For example, the control (GS), received a mean preference score of 0.06 (high preference) from one evaluator, and a mean of 7.8 (low preference) from a different evaluator (Figure 3-12). Similarly, DDSH had a mean score of 3.1 (high preference) from one evaluator, and a mean of 8.9 (low preference) from another evaluator (Figure 3-12). These results illustrate the often extreme variability in the perception of flavor quality for chocolate samples between evaluators. However, current results do not include the sensory evaluations from Fall 2018, and are therefore relatively inconclusive.

3.7 Conclusions

The results of this study show that heat source affects drying rate, pH, color attributes, and flavor intensity scores of cacao beans that were processed in Hawai'i over a 1.5-year time frame. Artificial methods, including ODC and DDC, had the fastest drying rates relative to the control. Natural methods, such as SDKC, and SDKS, also reduced drying times but at lower rates than artificial methods. Mean pH values for both the testa and cotyledon were shown to increase throughout the drying period for each treatment. Natural drying methods, SDKS, and SDPC, had the highest pH values, whereas artificial methods, such as ODC and DDC, had the lowest. There was shown to be a positive linear relationship between drying time and pH for both cotyledon and testa. For color

attributes, an artificial drying method (ODC) had the lowest C* and H* values, but there were no differences among treatments for L* values.

Sensory evaluations showed that an intermittent artificial treatment (ODS), had the highest fresh fruit intensity compared to the control (GS), which scored the lowest. For herbal/floral intensities, the control scored highest, and an intermittent natural treatment (SDKS) scored lowest. However, there were no differences in overall preference scores between treatments, although results from the sensory evaluation exclude data from Fall 2018 because of timing issues. Once results from the final round of flavor evaluations are included, there may be more noticeable differences in preference scores between treatments. Because the data for overall preference ratings are still inconclusive, recommendations of specific drying treatments, for cacao growers that aim to maximize quality, cannot be made with certainty. Similarly, the cost of labor and infrastructure for each drying treatment should be examined more thoroughly before recommendations to growers can be made.

at treatments were conducted

at treatments were conducted

Protocol	Dates	Sample size
daily until dry.	10/2017 - 12/2018	11
daily until dry.	10/2018 - 12/2018	3
daily, and then sealed in airtight containers at night. Repeated until dry.	10/2018 - 12/2018	3
daily until dry.	10/2017 - 12/2018	11
rd drying protocol* until dry.	10/2017 - 12/2018	11
daily until dry.	10/2018 - 12/2018	3
of sun for the first three days, and full sun every day after until dry.	10/2018 - 12/2018	3
rd drying interval daily at 57°C until dry.	10/2017 - 12/2018	11
rd drying protocol* until dry.	10/2017 - 12/2018	11
rs of drying with a 22-hour rest period daily until dry.	10/2017 - 12/2018	11
rd drying interval daily at 30% RH until dry.	11/2017 - 12/2018	10
and decreased in 10% increments every 24 hours until dry.	11/2017 - 12/2018	10

rd drying, exposure type, specific drying protocol, dates that treatments were conducted, and total sample size.

t day, four hours the second day, six hours the third day, and then full sun every day after until complete.

Table 3-4 – Color attributes of beans among drying treatments

Sample ID	L*	C*	H*
DDC	33.30 ± 1.67 ^{ns}	14.03 ± 1.22 ^{ab}	45.16 ± 2.01 ^{ab}
DDSD	33.40 ± 4.01 ^{ns}	13.12 ± 1.80 ^b	52.25 ± 2.62 ^a
ODC	30.69 ± 2.53 ^{ns}	11.45 ± 0.18 ^b	42.88 ± 1.98 ^b
ODS	31.30 ± 2.06 ^{ns}	13.01 ± 0.45 ^b	46.28 ± 3.52 ^{ab}
ODT	31.57 ± 2.12 ^{ns}	12.48 ± 0.71 ^b	47.09 ± 2.77 ^{ab}
SDKC	31.01 ± 1.10 ^{ns}	13.81 ± 0.41 ^b	46.58 ± 2.89 ^{ab}
SDKS	32.42 ± 2.98 ^{ns}	12.68 ± 0.79 ^b	48.05 ± 1.24 ^{ab}
SDKWC	30.79 ± 1.68 ^{ns}	18.59 ± 0.54 ^a	46.63 ± 2.71 ^{ab}
SDKWS	31.22 ± 3.09 ^{ns}	15.16 ± 1.11 ^{ab}	48.07 ± 3.04 ^{ab}
SDP	30.91 ± 5.40 ^{ns}	13.53 ± 2.02 ^b	50.44 ± 0.09 ^{ab}
SDPC	38.52 ± 3.14 ^{ns}	13.62 ± 0.82 ^{ab}	54.97 ± 1.13 ^a
SDPS	36.73 ± 4.10 ^{ns}	15.15 ± 0.97 ^{ab}	50.47 ± 1.34 ^{ab}

Values are means ± SEM, n = 3 per treatment group.

Means in a column that do not share a common superscript letter differ (<0.05) as analyzed by one-way ANOVA and post hoc.

Sample ID list: Dehumidification Dry Constant (DDC), Dehumidification Dry Step-Down (DDSD), Oven Dry Constant (ODC), Oven Dry Standard (ODS), Oven Dry Trinidad (ODT), Sun Dry Kainaliu Constant (SDKC), Sun Dry Kainaliu Standard (SDKS), Sun Dry Kawaihae Constant (SDKWC), Sun Dry Kawaihae Standard (SDKWS), Sun Dry Pāpai‘kou (SDP), Sun Dry Pepe‘ekea Constant (SDPC), Sun Dry Pepe‘ekea Standard (SDPS).

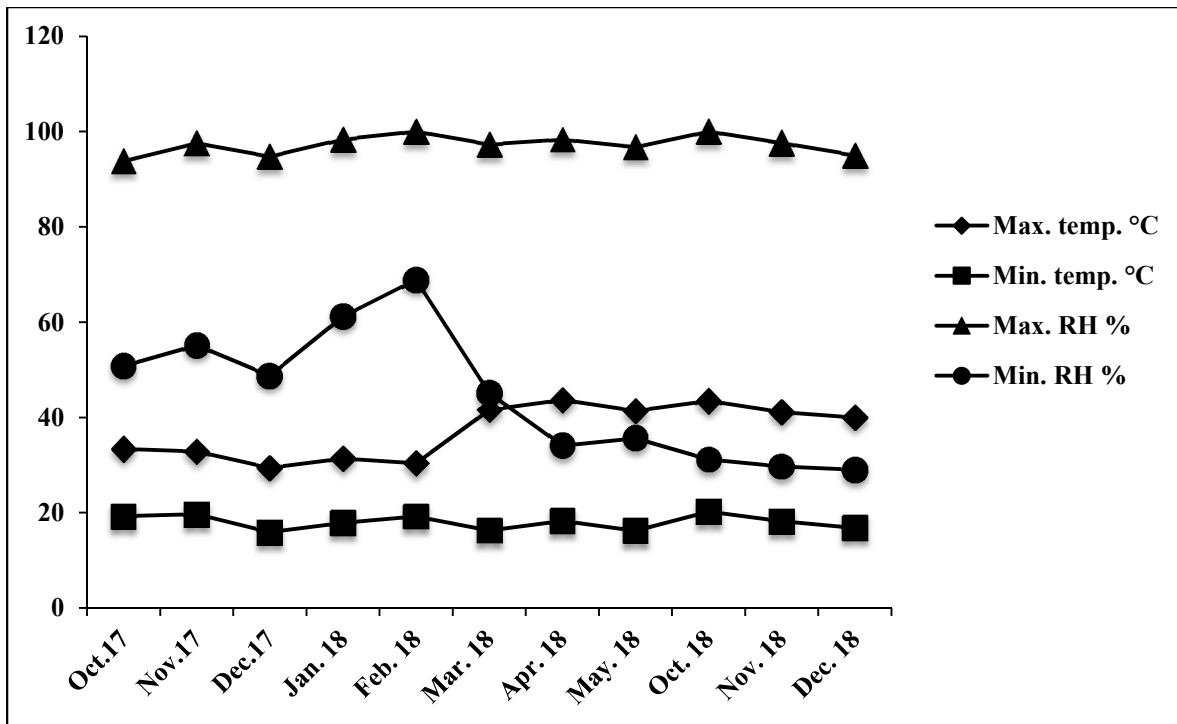


Figure 3-1– Mean maximum and minimum values for temperature (°C) and relative humidity (%) within the drying greenhouse located in Pāpai'kou. Values are averaged by monthly drying periods.

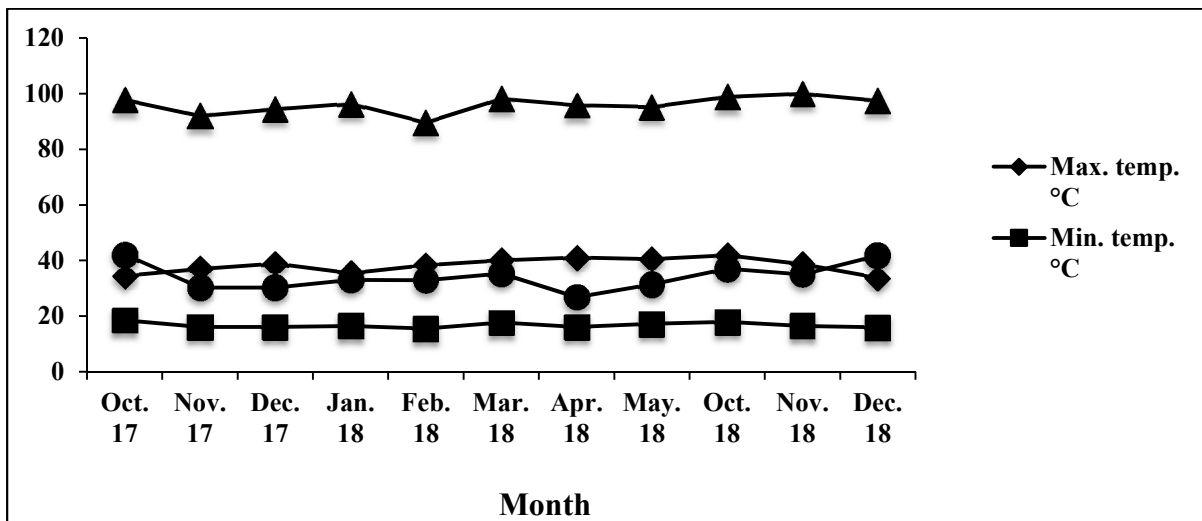


Figure 3-2 – Mean maximum and minimum values for temperature (°C) and relative humidity (%) within the drying greenhouse located in Kainaliu. Values are averaged by monthly drying periods.

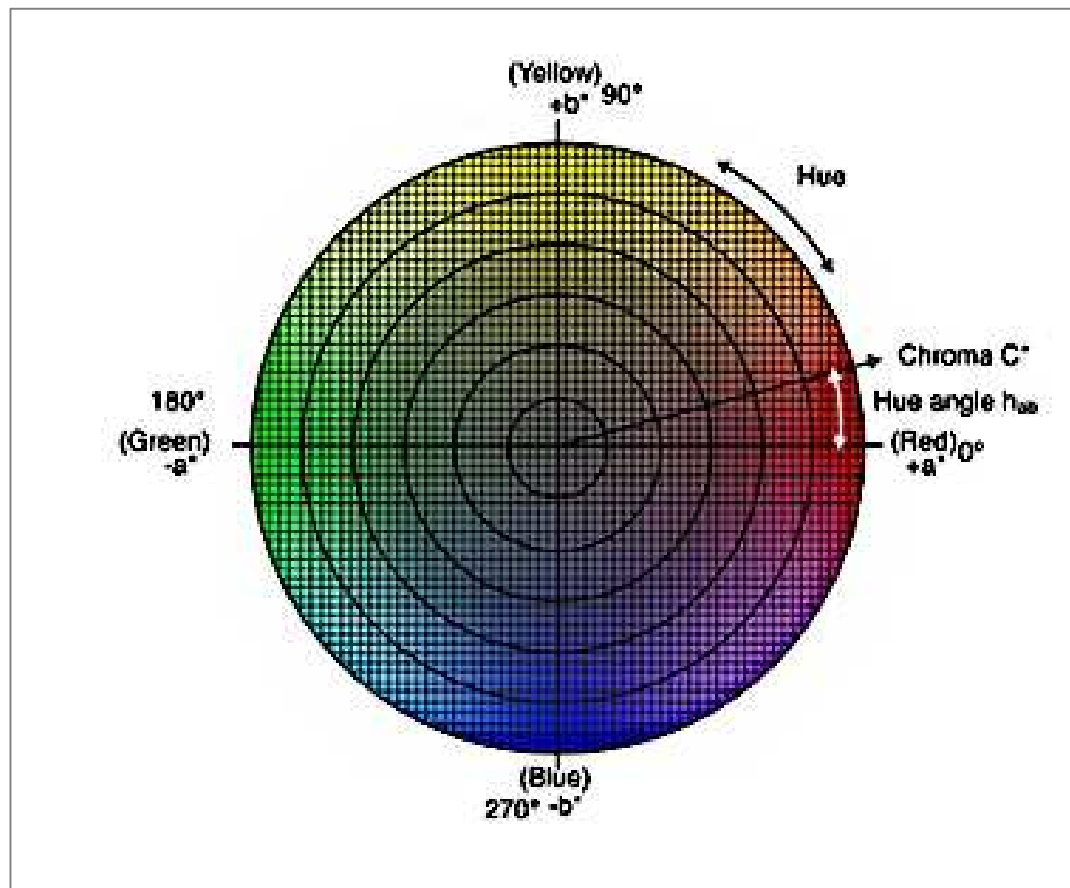


Figure 3-3 – L*C*H* Color Space Model.
 L^* represents lightness, C^* represents chroma, H^* represents hue angle.

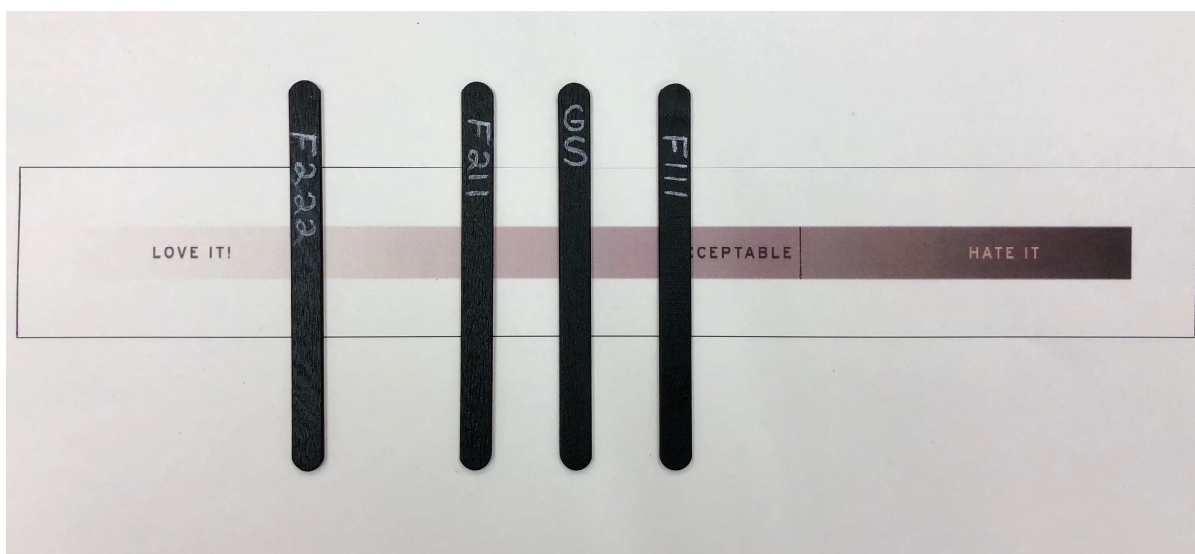


Figure 3-4 – Example of flavor evaluation results using the Love it/Acceptable/Hate it scale.

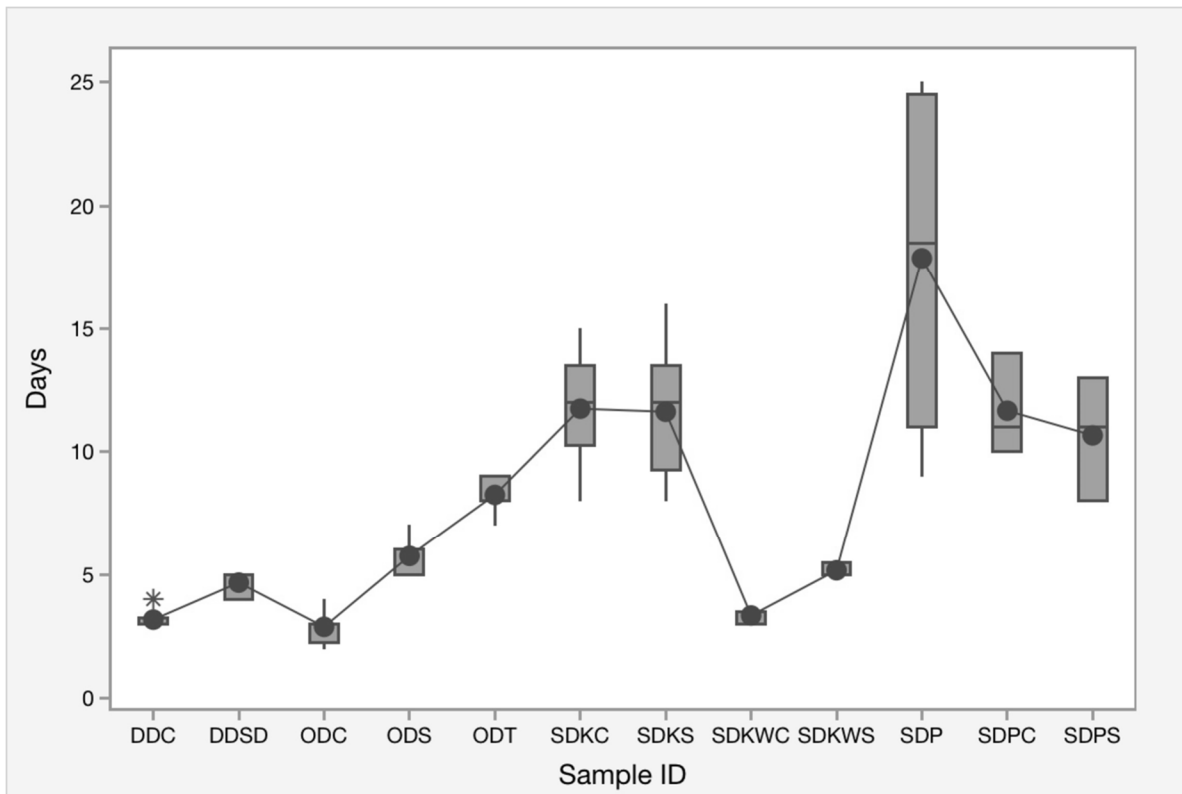


Figure 3-5– Summary statistics for drying rates between all treatments. Circles represent means, center lines represent medians, lower and upper ranges of boxes represent first and third quartiles, respectively. Whiskers represent minimum and maximum values. Asterisks represent outliers.
Sample ID list: Dehumidification Dry Constant (DDC), Dehumidification Dry Step-Down (DDSD), Oven Dry Constant (ODC), Oven Dry Standard (ODS), Oven Dry Trinidad (ODT), Sun Dry Kainaliu Constant (SDKC), Sun Dry Kainaliu Standard (SDKS), Sun Dry Kawaihae Constant (SDKWC), Sun Dry Kawaihae Standard (SDKWS), Sun Dry Pāpai‘kou (SDP), Sun Dry Pepe‘ekeo Constant (SDPC), Sun Dry Pepe‘ekeo Standard (SDPS).

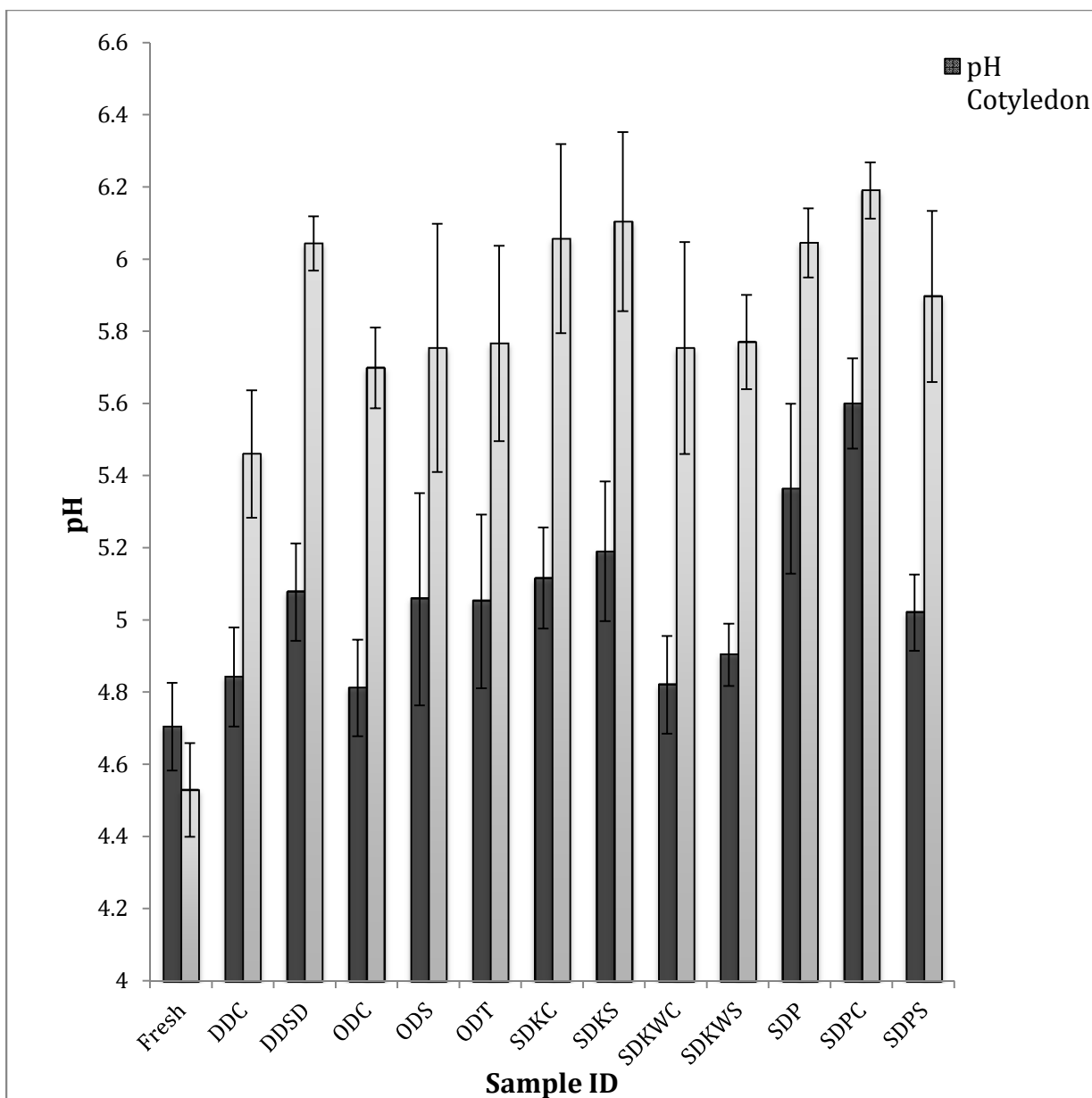


Figure 3-6 – Mean pH values of fresh and dried beans (cotyledons and testa). Error bars represent Standard Deviation.

Sample ID list: Dehumidification Dry Constant (DDC), Dehumidification Dry Step-Down (DDSD), Oven Dry Constant (ODC), Oven Dry Standard (ODS), Oven Dry Trinidad (ODT), Sun Dry Kainaliu Constant (SDKC), Sun Dry Kainaliu Standard (SDKS), Sun Dry Kawaihae Constant (SDKWC), Sun Dry Kawaihae Standard (SDKWS), Sun Dry Pāpai'kou (SDP), Sun Dry Pepe'ekeo Constant (SDPC), Sun Dry Pepe'ekeo Standard (SDPS).

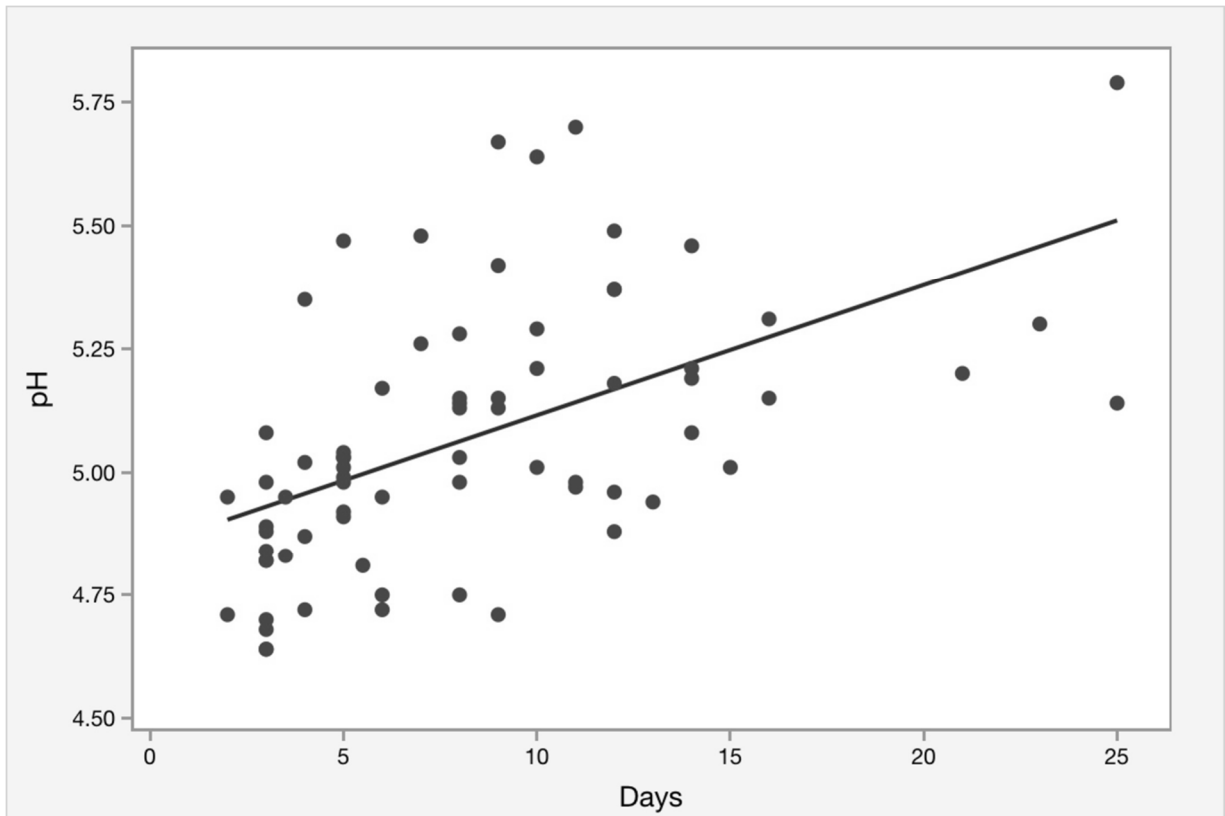


Figure 3-7 -Scatterplot with best fitting least squares regression line showing relationship between pH of cotyledon and days to drying at 7-7.5% moisture content.

$$y = 4.83797x + 0.028551$$

$$R^2 = 28.56\%$$

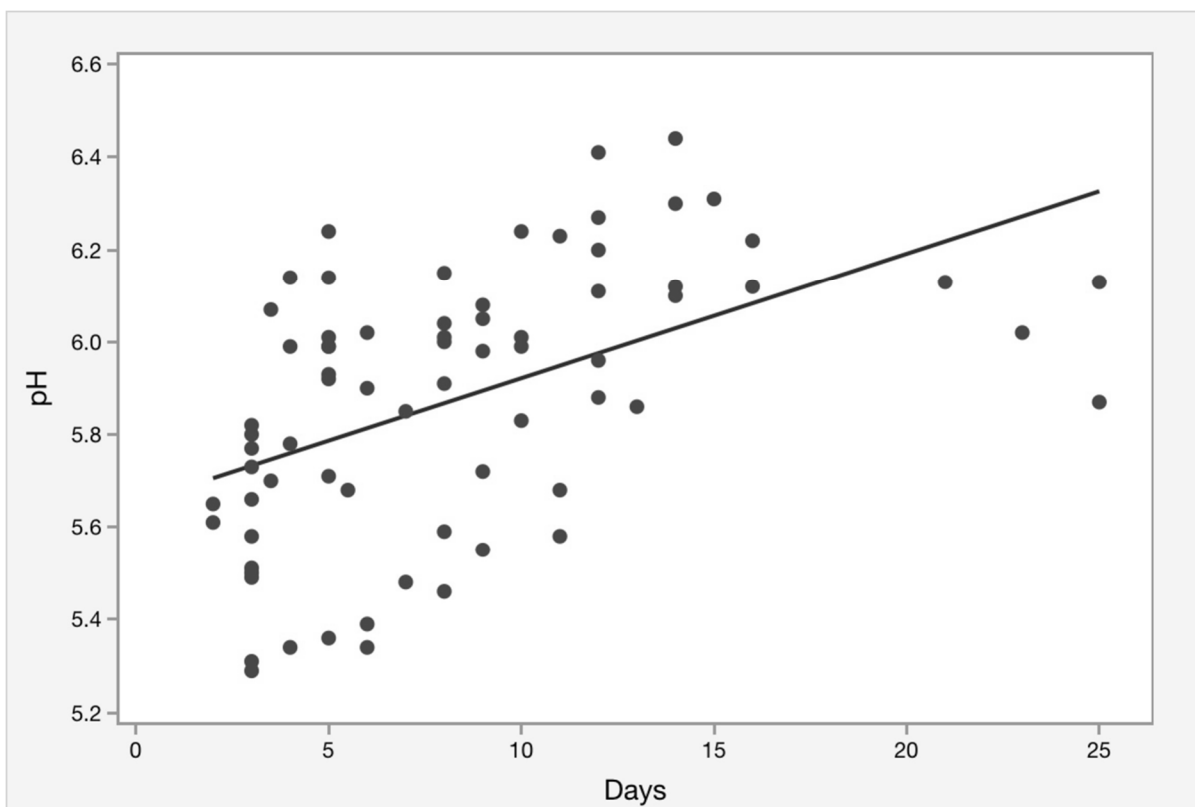


Figure 3-8 - Scatterplot with best fitting least squares regression line showing relationship between pH of testa and days to drying at 7-7.5% moisture content.
 $y = 5.65168x + 0.026995$
 $R^2 = 24.92\%$

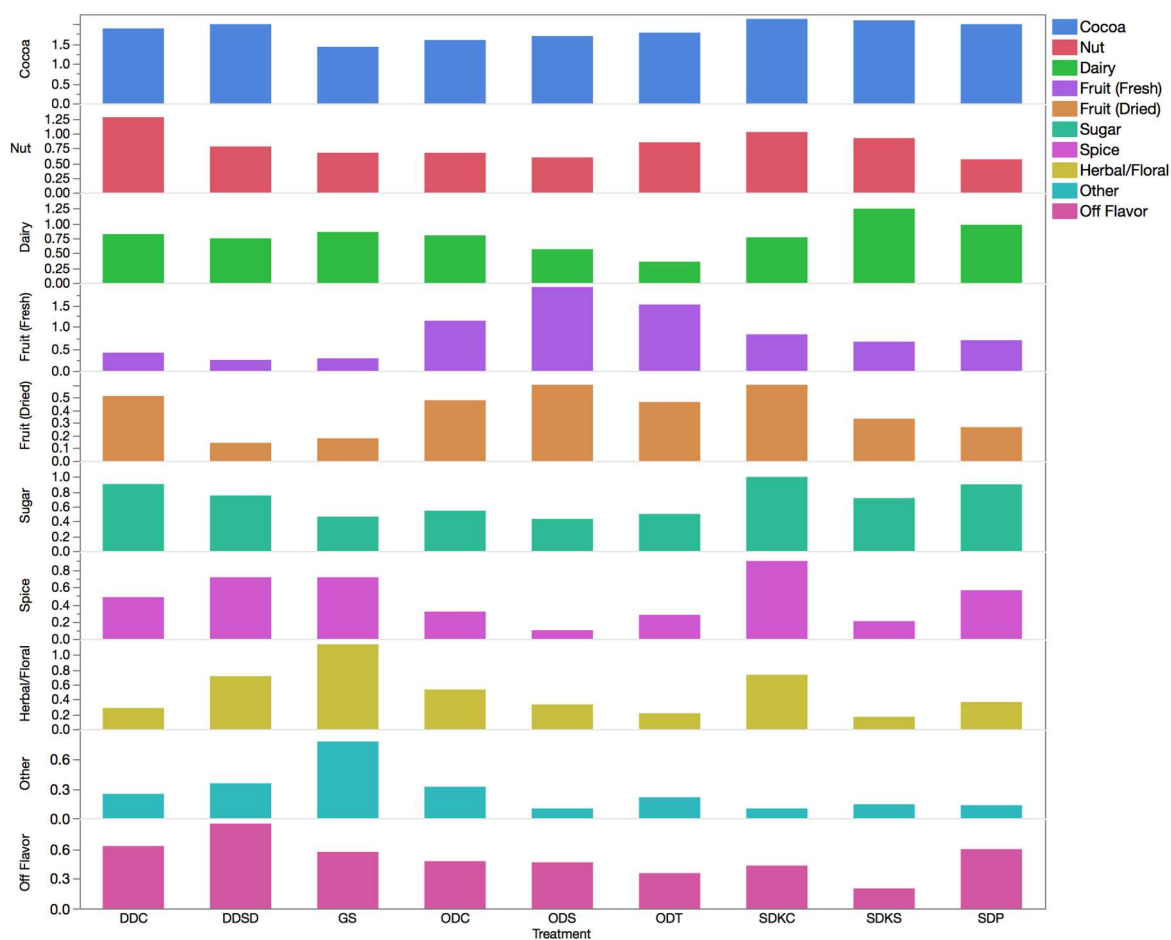


Figure 3-9 – Mean values for flavor intensity scores between drying treatments. Flavor intensity scale is from 0-3 (0 = not present, 1 = faint, but present, 2 = present, but not main note, 3 = dominant). Sample ID list: Dehumidification Dry Constant (DDC), Dehumidification Dry Step-Down (DDSD), Ghana Standard* (GS), Oven Dry Constant (ODC), Oven Dry Standard (ODS), Oven Dry Trinidad (ODT), Sun Dry Kainaliu Constant (SDKC), Sun Dry Kainaliu Standard (SDKS), Sun Dry Pāpai‘kou (SDP). *Ghanaian cacao is the standard by which all cacao is measured (Afoakwa, 2016).

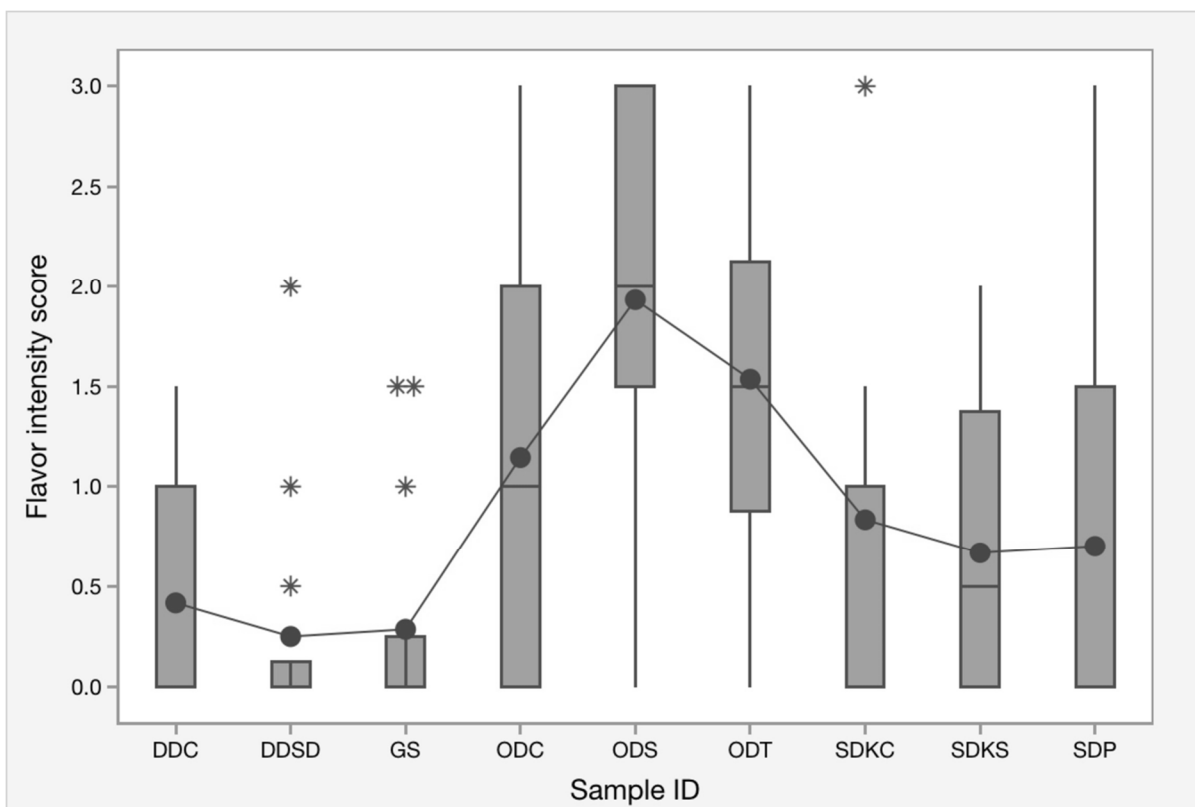


Figure 3-10 - Summary statistics for fresh fruit intensity scores between all treatments. Circles represent means, center lines represent medians, lower and upper ranges of boxes represent first and third quartiles, respectively. Whiskers represent minimum and maximum values. Asterisks represent outliers.

Sample ID list: Dehumidification Dry Constant (DDC), Dehumidification Dry Step-Down (DDSD), Ghana Standard* (GS), Oven Dry Constant (ODC), Oven Dry Standard (ODS), Oven Dry Trinidad (ODT), Sun Dry Kainaliu Constant (SDKC), Sun Dry Kainaliu Standard (SDKS), Sun Dry Pāpai'kou (SDP).

*Ghanaian cacao is the standard by which all cacao is measured (Afoakwa, 2016).

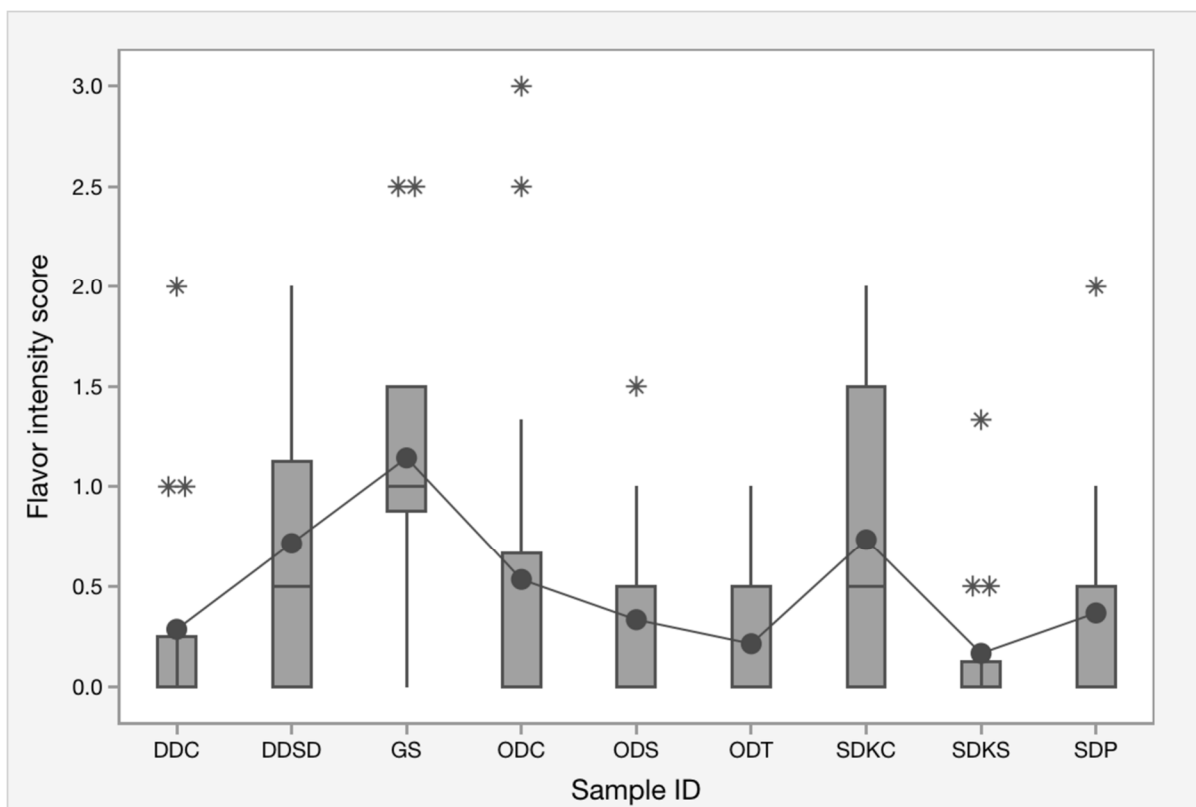


Figure 3-11 – Summary statistics for herbal/floral intensity scores between all treatments. Circles represent means, center lines represent medians, lower and upper ranges of boxes represent first and third quartiles, respectively. Whiskers represent minimum and maximum values. Asterisks represent outliers.

Sample ID list: Dehumidification Dry Constant (DDC), Dehumidification Dry Step-Down (DDSD), Ghana Standard* (GS), Oven Dry Constant (ODC), Oven Dry Standard (ODS), Oven Dry Trinidad (ODT), Sun Dry Kainaliu Constant (SDKC), Sun Dry Kainaliu Standard (SDKS), Sun Dry Pāpai'kou (SDP).

*Ghanaian cacao is the standard by which all cacao is measured (Afoakwa, 2016).

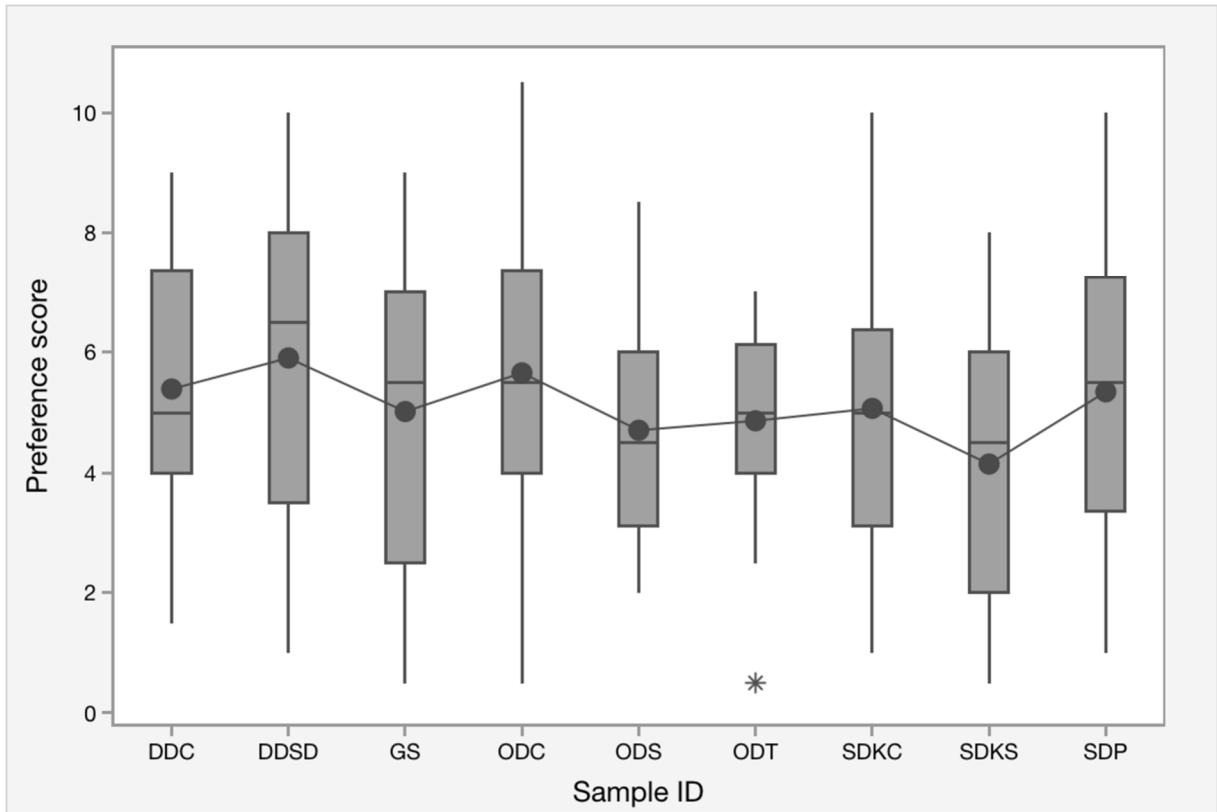


Figure 3-12 – Summary statistics for overall preference scores between all treatments. Circles represent means, center lines represent medians, lower and upper ranges of boxes represent first and third quartiles, respectively. Whiskers represent minimum and maximum values. Asterisks represent outliers. Sample ID list: Dehumidification Dry Constant (DDC), Dehumidification Dry Step-Down (DDSD), Ghana Standard* (GS), Oven Dry Constant (ODC), Oven Dry Standard (ODS), Oven Dry Trinidad (ODT), Sun Dry Kainaliu Constant (SDKC), Sun Dry Kainaliu Standard (SDKS), Sun Dry Pāpai‘kou (SDP). *Ghanaian cacao is the standard by which all cacao is measured (Afoakwa, 2016).

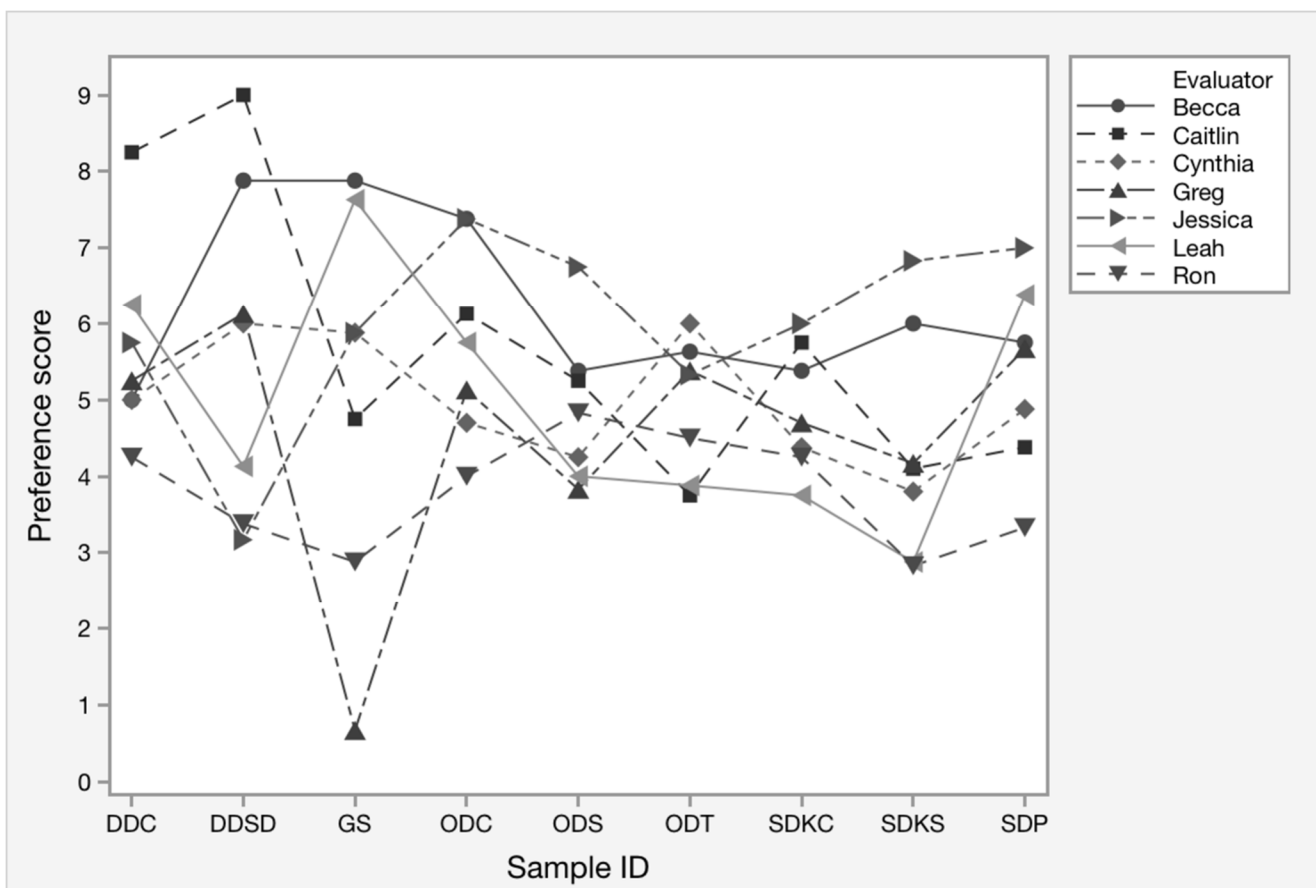


Figure 3-13 – Mean overall preference scores among drying treatments for each evaluator.
Sample ID list: Dehumidification Dry Constant (DDC), Dehumidification Dry Step-Down (DDSD), Ghana Standard* (GS), Oven Dry Constant (ODC), Oven Dry Standard (ODS), Oven Dry Trinidad (ODT), Sun Dry Kainaliu Constant (SDKC), Sun Dry Kainaliu Standard (SDKS), Sun Dry Pāpai‘kou (SDP).
*Ghanaian cacao is the standard by which all cacao is measured (Afoakwa, 2016).

3.9 References

Afoakwa, E.O., A. Paterson, M. Fowler, and A. Ryan. 2008. Flavor information and character in cocoa and chocolate: A critical review. *Critical Reviews in Food Science and Nutrition* 48:840-857.

Afoakwa, E.O. 2016. *Cocoa Production and Processing Technology*. Boca Raton, FL: Taylor & Francis Group.

Amoa-Awua, W., Dahl, M., Takrama, J.F., Olaiya, A., Ban-Koffi., Jakobsen, M. 2007a. Quality manual for production and primary processing of cocoa. EC INCO Project document (Development of biochemical and molecular markers for determining quality assurance in the primary processing of cocoa in West Africa). Available at CSIR-Food Research Institute, Accra, Ghana, and Department of Dairy and Food Science, Faculty of Life Sciences, University of Copenhagen, Denmark.

Asiedu, J.J. 1989. *Processing Tropical Crops: A Technological Approach*. Macmillan Press Limited, London, pp. 24-41.

Bonaparte, A.Z. 1995. Solar drying of cocoa beans (*Theobroma cacao*) in St Lucia. MSc thesis, McGill University, Quebec, Canada.

Dahl, M.W. 2006. Development of quality management system for the primary processing of cocoa. MSc thesis, Royal Veterinary and Agricultural University, Copenhagen, Denmark.

De Vos, L. 1956. Artificial drying of cocoa. Bull. 73, Landouwproef Station in Suriname.

Fagunwa, A. O., Koya, O. A. and Faborode, M.O. 2009. Development of an intermittent solar dryer for cocoa beans. *Agricultural Engineering International: CIGR Journal*.

Ghosh, B.N. 1972. Engineering aspects of cocoa drying in Brazil. *Revista Theobroma*, 2: 23-37.

Hart, J. H. 1900. *Cacao. A treatise on the cultivation and curing of 'cacao'* (2nd edn.) Mirror, Port-of-Spain, Trinidad.

Hashim, P., Sclamat, J., Muhammad, S.K.S., Ali, A. 1998. Changes in free amino acid peptide-N, sugar and pyrazine concentration during cocoa fermentation. *Journal of Science, Food and Agriculture*, 78:535-542.

Hii, C.L., Abdul Rahman, R., Jinap, S. and Che Man, Y.B. 2006. Quality of cocoa beans dried using a direct solar dryer at different loadings, *Journal of the Science of Food and Agriculture*, 86 (8), 1237-1243.

Hii, C.L., Law, C.L., Cloke, M., Suzannah, S. 2011. Improving Malaysian cocoa quality through the use of dehumidified air under mild drying conditions, *J. Sci. Food. Agric.* 91:239-246.

Hii, C.L., Law, C.L., Suzannah, S. 2012. Drying kinetics of the individual layer of cocoa beans during heat pump drying, *Journal of Food and Engineering*, 108: 276-282.

Jinap, S., Thien, J., Yap, T.N. 1994. Effect of drying on acidity and volatile fatty acids content of cocoa beans. *Journal of the Science of Food and Agriculture*, 65:67-75.

Kumi, W.O. 2005. Microbial species involved in heap and tray fermentation of cocoa beans in Ghana. MPhil thesis, University of Ghana, Accra, Ghana.

Mujaffaer, S., Sukha, D. A., Ramroop, A. 2017. Comparison of the drying behavior of fermented cocoa (*Theobroma cacao* L.) beans dried in a cocoa house, greenhouse and mechanical oven. Cocoa Research Centre, The University of the West Indies, St. Augustine, Trinidad.

Oke D.O., and Omotayo K.F. (2012). Effect of forced-air artificial intermittent drying on cocoa beans in outh-Western Nigeria. Department of Agriculture and Bio-environmental Engineering. School of Engineering. Nigeria.

Powell, B.D. 1982. The quality of cocoa beans – The needs of the manufacturer. In *Proceedings of the 8th International Cocoa Research Conference held in Cartanaga, Colombia*, pp. 755 – 758.

Schwan, R. F. and Wheals, A. E. 2004. The microbiology of cocoa fermentation and its role in chocolate quality. *Crit. Rev. Food Sci. Nutr.* 44:205-221.

Shelton, B. 1967. Artificial drying of cocoa beans. *Tropical Agriculture*, 44:125-32.

Sukha, D. A. 2003. Primary processing of high quality Trinidad and Tobago cocoa beans-targets, problems, options. Cocoa Research Unit, University of the West Indies, St. Augustine Trinidad.

Takrama, J.F., P.C. Aculey, and F. Aneani. 2006. Fermentation of cocoa with placenta: A scientific study. In *Proceedings of 15th International Cocoa Research Conference; Costa Rica*. Volume II, pp. 1373-1379.

Thompson, S.S., Miller, K.B., Lopez, A.S. 2001. Cocoa and coffee. In Doyle, M.J., Beuchat, L.R., Montville, T.J. (Eds.). *Food Microbiology – Fundamentals and frontiers*. ASM Press, Washington, DC, pp. 721-733.

Urquhart, D.H. 1961. Cocoa, 2nd edn. Western Printing Services Ltd., Bristol, Great Britain.

Wood, G.A.R. 1975. Cocoa, 3rd edn. Tropical Agricultural Series, Longman Inc., New York.

Wood, G.A.R., and R.A. Lass. 1985. Cocoa, 4th edition. London, UK: Longman Group.

CHAPTER 4

CONCLUSIONS

4.1 Fermentation

Three fermentation protocols, relating to the timing of turning the fermentation mass, were evaluated through treatment application from September 2017 until April 2018. The treatment that was turned least frequently (F222) scored most favorably in the sensory evaluations, although the considerable levels of mold infestation that emerged in the bottom layers of the fermentation box makes this protocol inadvisable for growers. This treatment produced beans that were more acidic than other treatments, which is generally associated with undesirable flavor, but did not appear to affect sensory evaluation results. F211, the treatment with an intermediate frequency of turning, had a slightly less favorable preference score, but displayed no signs of mold contamination during fermentation and could therefore be a possible recommendation for growers in Hawai‘i. The treatment that was turned most frequently (F111) showed certain positive fermentation traits, including the rate of temperature increase, which could be of importance considering that fermentations in Hawai‘i often take longer to reach critical temperatures than in other regions worldwide. However, the relatively poor results it received in the sensory evaluations makes this protocol inadvisable for use in Hawai‘i.

It is possible that the long 48-hour intervals between turning contributed to spoilage in the bottom layers of the F222 treatment, especially during the last 2-3 days of fermentation when populations of molds and spore formers are at their peak. The sustained periods of low temperatures at the bottom of the fermentation mass may have

provided an ideal environment for these microorganisms to proliferate. Integrating beneficial aspects from each fermentation treatment could be the subject of future research. For example, it may be advisable to use the vigorous early turning interval of F111 in combination with the longer resting periods of F222, and then finishing the ferment with another set of 24-hour turns to increase aeration and temperature during the final stages of fermentation. Integrating these various turning intervals has potential to maximize some of the positive traits from each protocol, while potentially mitigating the defects associated with them.

4.2 Drying

Application of 12 different drying trials was evaluated over a 1.5 year period. There were no differences in overall preference scores between treatments, although results from the sensory evaluation exclude data from Fall 2018 because of timing issues. Once results from the final round of flavor evaluations are included, there may be more noticeable differences in preference scores between treatments. Other post-harvest parameters, including drying rate, pH, and color attributes, were affected by treatment. For example, the control, SDP, took by far the longest to dry, compared to constant artificial treatments (ODC and DDC), both of which had the lowest pH values. Two natural drying treatments (SDPC and SDPS), although only replicated in Fall 2018, may be promising alternative methods of sun drying on the eastern regions of Hawai'i Island. Both treatments had significantly quicker drying times than the control, which could be attributed to the increased airflow of that drying system. However, a longer-term study is needed to

evaluate the effect of environmental conditions, such as airflow, on drying rates and quality parameters. For example, the open-air drying conditions of SDPC and SDPS could potentially lead to slower drying under more extreme weather conditions due to increased exposure to the environment. Results from the sensory evaluation are not currently included for this treatment, so the impact on flavor is also unknown.

Flavor intensity scores differed between treatments for certain categories, including fresh fruit and herbal/floral. However, these flavor groups showed no correlation to overall preference ratings. These results exemplify the variability in perceptions of preference between evaluators. For example, some evaluators associated the high levels of fresh fruit intensity in an intermittent artificial treatment (ODS) as desirable, whereas others rated it poorly. Similarly, the high ratings of herbal/floral in the control, or the marginally higher cocoa flavors of SDKS and SDKC, are subjective flavor groups that do not necessarily correspond with categorical markers of quality, but rather on the individual preferences of the evaluator. This component of sensory evaluation has not been studied thoroughly, and is a necessary inclusion in further research, especially as major segments of the industry move away from bulk chocolate production, with its limited and homogenized flavor characteristics, into the more complex and nuanced platform of specialty chocolate.